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Studies on the role of leukotrienes in murine allergic and irritant contact dermatitis

T. ROSENBACH, M. CSATÒ AND B.M. CZARNETZKI

Department of Dermatology, University Clinics, Münster, West Germany

Accepted for publication 13 July 1987

SUMMARY

A specific peptidoleukotriene receptor antagonist, Ro 23-3544, was tested for its efficacy in modulating DNFB-induced allergic and croton oil-induced irritant contact dermatitis in mouse ears. Treatment shortly after elicitation of the dermatitis, and for up to 5 days thereafter, was moderately effective in suppressing DNFB-induced ear swelling in a dose-dependent fashion. Daily pre-treatment of the ears for 1 week caused a more marked reduction of DNFB-induced ear swelling during the first 48 h after elicitation. No reduction, but rather an increase in ear swelling was observed with croton oil-induced dermatitis. These results indicate that peptidoleukotrienes play a role in the early stages of elicitation of murine allergic, but not irritant contact dermatitis and that a specific receptor antagonist can only partially reverse the effect of peptidoleukotrienes once the dermatitis is established.

In recent years, there has been a major effort to develop new drugs against a group of highly potent mediators of inflammation derived from arachidonic acid (AA) via two different pathways. One of these pathways leads via the cyclo-oxygenase enzyme to prostaglandins (PGs) and thromboxanes, and the other causes the lipoxygenase-dependent generation of leukotrienes (LTs) and monohydroxyeicosatetraenoic acids (HETEs) (for review, see Sammuelsson[1]). All these substances are potentially active as mediators of cutaneous inflammation: LTB_4 causes leukocyte migration, aggregation, and adherence[2], the peptidoleukotrienes LTC_4, LTD_4, and LTE_4 increase vascular permeability, induce smooth muscle contraction, and cause microvascular dilatation,[3] and PGE_2 acts as a vasodilator which potentiates synergistically the action of other mediators on blood vessels.[4]

Prostaglandins and leukotrienes are elevated in inflamed tissue, particularly in dermatological diseases such as psoriasis,[5,6] atopic eczema, bullous pemphigoid[8] and contact dermatitis.[9] The pathogenetic role or the contribution of AA-derived mediators in the maintenance of these diseases is, however, unclear.

Correspondence: Professor Dr B.M. Czarnetzki, F. Hoffmann-La Roche & Co. Ltd., Clinical Research Dermatology, CH-4002 Basle, Switzerland.

One possible approach to the clarification of this question would be the use of specific enzyme inhibitors or receptor antagonists. Such agents have been useful in elucidating the role of prostaglandins in inflammatory diseases. Non-toxic inhibitors of leukotriene generation and function with a good bioavailability have been developed only very recently.

One such inhibitor is Ro 23-3544, a peptidoleukotriene receptor antagonist which effectively reduces LTC_4- and antigen-induced bronchoconstriction in the guinea pig.[10] The inhibition by the antagonist is specific for peptidoleukotrienes since histamine- or platelet activating factor (PAF)-induced bronchoconstriction is not affected.[10] This novel antagonist was, therefore, chosen to examine the possible role of peptidoleukotrienes in murine contact dermatitis, induced by dinitrofluorobenzene (DNFB) or croton oil. The choice of this model seemed to be particularly relevant in view of the fact that elevated tissue levels of peptidoleukotrienes have been demonstrated previously in murine contact dermatitis.[11]

METHODS

Female BALB/c mice were sensitized twice with 15 μl of 0·5% DNFB in acetone/olive oil (4:1) on the razor shaved abdomen.[12] Two days later, allergic contact dermatitis was elicited by painting both ears with 20 μl of 0·3% DNFB (Sigma, Munich, F.R.G.). Irritant dermatitis was induced by a single application of 0·5% croton oil (Sigma, Munich, F.R.G.) in acetone/olive oil (4:1).

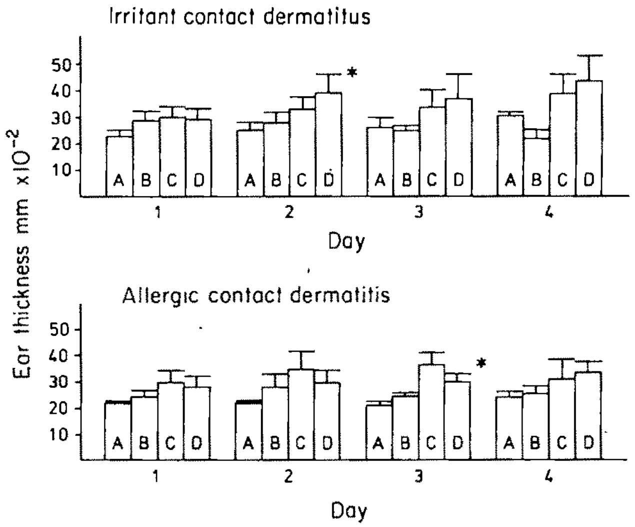

FIGURE 1. Thickness of mouse ears after induction of irritant contact dermatitis with croton oil (CO) or allergic contact dermatitis with DNFB, with or without subsequent application of 5% Ro 23-3544 in PEG 400 or PEG 400 alone.

A: Ro 23-3544 on normal control ears
B: CO or DNFB
C: CO or DNFB + PEG 400
D: CO or DNFB + Ro 23-3544
Bars are means ± SD of groups of 10 mice.
*D significantly different from C, $P < 0{\cdot}05$.

Ro 23-3544 (from Fa. Hoffmann-La Roche, Nutley, U.S.A.) was dissolved in polyethylene glycol (PEG) 400 and in some experiments in acetone/olive oil, 4:1. Concentrations of 0·1, 0·5, 1·0 and 5·0% of the antagonist were applied to one of the pretreated ears 1 h after elicitation and at 24 h intervals on the following 4 days. One series of animals was treated with applications of 5·0% Ro 23-3544 three times daily. Normal and elicited ears were treated with Ro 23-3544 or

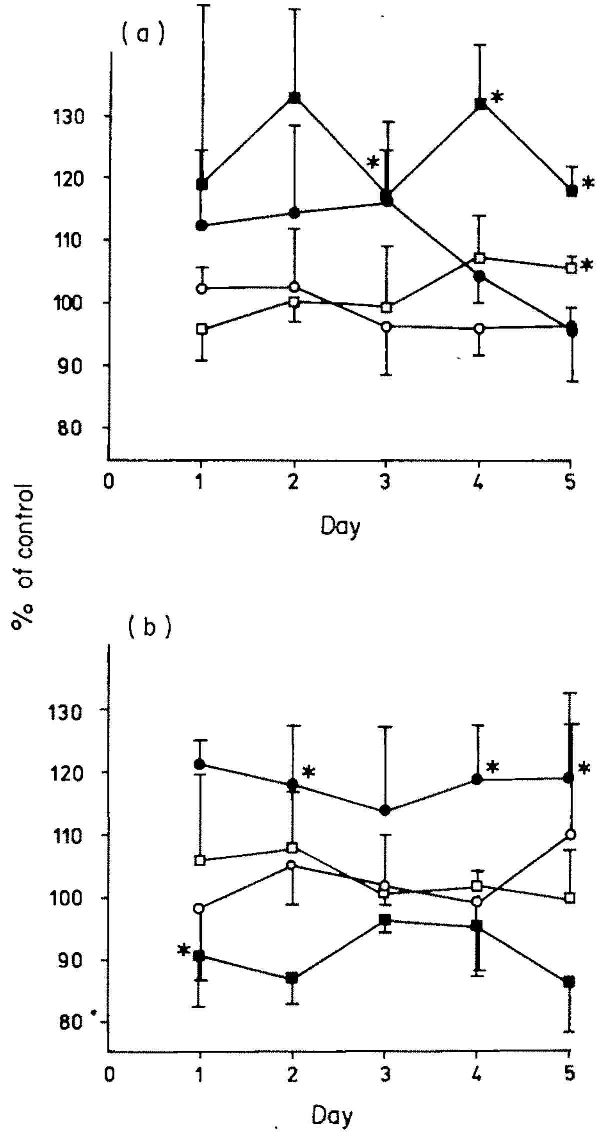

FIGURE 2. Time course of ear swelling in (a) irritant contact dermatitis and (b) allergic contact dermatitis after treatment with Ro 23-3544, 0·1% once daily (□), 1·0% once daily (○), 5·0% once daily (■), or 5·0% three times daily (●). Results are expressed as a percentage of control ears treated with PEG 400 alone after elicitation of dermatitis. Points are means ± SD of groups of four mice. *Significantly different from control ($P < 0{\cdot}05$).

with PEG 400 alone as controls. In some experiments, ears were pre-treated with 5·0% Ro 23-3544 once daily for 1 week before elicitation of the dermatitis, with no additional treatment thereafter.

After each treatment, the ears were dried with a hair dryer. Ear thickness was measured with a spring loaded micrometer prior to, and 24 h after each treatment for up to 5 days.

Results are based on groups of four to 10 mice each. Statistical analysis was carried out using an unpaired Student's *t*-test to compare each set of experimental measurements with their respective controls measured on the same day.

RESULTS

In a first set of experiments, 5·0% Ro 23-3544 in PEG 400 was applied daily to one ear of animals with irritant or allergic contact dermatitis (Fig. 1). Ears of animals treated with the antagonist alone, with croton oil or DNFB alone, and with the vehicle PEG 400 alone, served as controls. The PEG 400 formed a thin film over the ears and increased the ear measurements artificially (compare columns B and C in Fig 1). The effect of Ro 23-3544 is, therefore, best evaluated by comparing groups C and D in Figure 1. In irritant contact dermatitis, the antagonist caused no reduction, but rather an increase in ear swelling ($P < 0{\cdot}05$ on day 2, otherwise the difference was not significant). In allergic contact dermatitis, the antagonist reduced the ear swelling on the first 3 days, with a significant reduction being reached on day 3 ($P < 0{\cdot}05$). Additional experiments with Ro 23-3544 in acetone/olive oil yielded similar results. Measurements of ears within 1 to 8 h after the first application of the irritant yielded highly variable results.

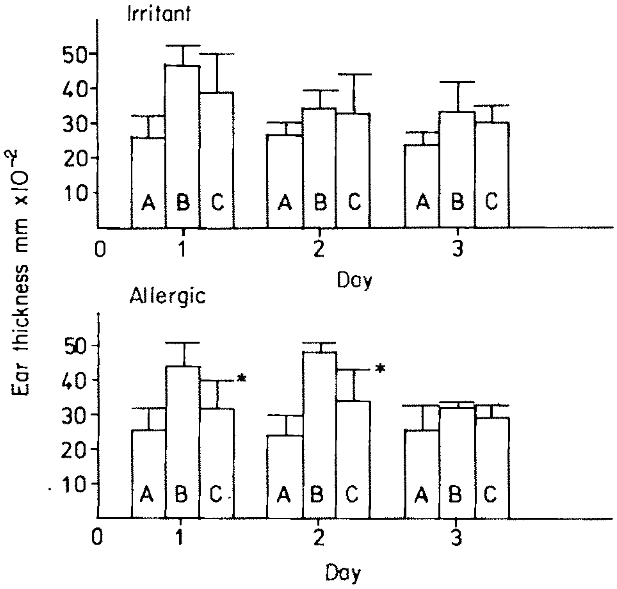

FIGURE 3. Thickness of mouse ears treated with Ro 23-3544 after induction of irritant contact dermatitis with croton oil (CO) or allergic contact dermatitis with DNFB, following daily application of Ro 23-3544 for 1 week.

A: Ro 23-3544 applied to normal control ears
B: CO or DNFB
C: CO or DNFB + Ro 23-3544

Bars are means ± SD for groups of 10 mice. *B significantly different from C ($P < 0{\cdot}05$).

In order to optimize the dose of the antagonist, the ears of the mice were treated with 5·0% Ro 23-3544 three times daily or with 5·0%, 1·0% or 0·1% once daily. The results for irritant and allergic contact dermatitis are shown in Figure 2. None of the treatment schedules caused a reduction of ear swelling in irritant dermatitis, and the increase in thickness with the 5·0% once daily treatment was significant on days 3–5 ($P < 0{\cdot}05$). In allergic contact dermatitis concentrations below 5·0% had no effect, there was a significant reduction in swelling on day 1 after one treatment with 5·0%, and a worsening of the swelling after applications three times daily.

The effects of pre-treatment of the ears with antagonist prior to elicitation of ear swelling are shown in Figure 3. With this method, the antagonist caused a reduction in ear swelling in allergic contact dermatitis on the first 2 days ($P < 0{\cdot}05$). There was no statistically signficant effect on irritant dermatitis.

DISCUSSION

The present data demonstrate that Ro 23-3544 is able to suppress allergic contact dermatitis during the early days of its elicitation. The effect is best when the skin is pre-treated for several days before elicitation. There is also a clear dose response relationship in that 0·1% and 1% of the antagonist were ineffective and treatment with a 5% solution three times daily caused an increase in the ear swelling.

In irritant dermatitis it was never possible to show an inhibitory effect by the antagonist, even with a suspension of the drug in acetone/olive oil to give improved penetration. The increased ear swelling observed here cannot be attributed solely to artificial thickening of the ear by the PEG 400 since PEG 400 treatment without the antagonist was used as a control.

The time course of the effect of the antagonist, which showed activity during the first 3 days of post-elicitation therapy and the optimal effects of the antagonist when administered prior to elicitation of allergic contact dermatitis are in accordance with a previous study in which arachidonate-dependent oedema was found to be maximal within 1 h and the responsiveness of the ears decreased over the following 48 h.[13] This oedema has been shown to be associated with increased PGE_2 and LTC_4 and LTD_4 synthesis and can be inhibited by 5-lipoxygenase and by cycloxygenase inhibitors, such as nordihydroguaretic acid, indomethacin or phenidone.[11,14] The later phase of inflammation most probably involves the infiltration of leukocytes, with the production of other mediators by these cells. Ro 23-3544 has some antichemotactic effects *in vitro* (B. M. Czarnetzki unpublished data), but these occur at high concentrations and are probably not significant *in vivo*.

The enhancement of irritant dermatitis by Ro 23-3544 is intriguing. Two explanations can be suggested. The antagonist might have a minor irritant effect on the skin which is ordinarily not evident but which would be synergistically enhanced by the elicitation of an irritant dermatitis with croton oil. Alternatively, irritant and allergic dermatitis involve different pathogenetic processes, and these might be affected differently by the antagonist. Against the first possibility is the fact that the drug is well tolerated in animal airways and human skin (B.M. Czarnetzki, unpublished), but the different properties of murine skin or the solvent might be important in the present results. Furthermore, a trend towards increased ear swelling with the antagonist in PEG 400 was evident from days 1 to 4.

There remains the possibility that different pathomechanisms in the two diseases account for the divergent responsiveness of the two models to the antagonist. One obvious difference in allergic compared with toxic eczema is the cytotoxic effect in the latter. Production of mediators requires, in most instances, an intact cell which is capable of biosynthesis, or enzymes that need certain optimal conditions for proper function. Such conditions might prevail in allergic, but not in irritant dermatitis. The observation of inhibition of lipoxygenase I in allergic dermatitis and an increased activity of this enzyme in irritant dermatitis in the guinea pig[15] would suggest that such divergence may well be real.

The present data also underline the fact that the pathogenesis and the consequent treatment of inflammatory diseases is complex and only rarely dependent on one single group of mediators. Nevertheless, new specific antagonists of mediators help to unravel the pathogenesis of these diseases and will perhaps pave the way to better therapeutic approaches.

ACKNOWLEDGMENTS

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Mucosal involvement in bullous and cicatricial pemphigoid. A clinical and immunopathological study

V.A.VENNING, P.A.FRITH*. A.J.BRON*, P.R.MILLARD† AND F.WOJNAROWSKA

Department of Dermatology, Slade Hospital, Oxford, * Oxford Eye Hospital, Walton St, Oxford and † Department of Histopathology, John Radcliffe Hospital, Oxford, U.K.

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SUMMARY

Thirty-six patients with bullous pemphigoid (BP) and 15 with cicatricial pemphigoid (CP) were studied for evidence of mucosal involvement both clinically and by direct immunofluorescence.

Twenty-one patients with BP and all those with CP had lesions of the mucous membranes. Eighteen patients with BP and 14 patients with CP had involvement of the oral mucosa. Lesions of the eyes, nose, pharynx, vulva and urethra also occurred in both groups. The lesions tended to be more widespread in CP patients. Twenty-one patients with BP and 14 with CP were examined by an ophthalmologist. Fine scarring of the tarsal conjunctiva was found in both groups, but also in elderly controls. However, symblepharon occurred in one patient with BP as well as in three patients with CP, but was not seen in a group of 20 controls.

Direct immunofluorescence (IF) studies showed linear deposition of IgG or C_3 or both in the basement membrane zone of the skin, oral mucosa and conjunctival mucosa in both groups. The frequency of positive immunofluorescence was higher in the conjunctiva than in the lip mucosa or skin in both BP and CP patients. The significance of these findings and the relationship between BP and CP is discussed.

Bullous pemphigoid (BP) and cicatricial pemphigoid (CP) share many features in common. Identical ultrastructural, as well as direct and indirect immunofluorescence (IF) findings bear witness to the similarities between these two diseases. Despite these similarities, CP is generally regarded as a clinical entity distinct from BP because of its predilection for the mucous membranes, less frequent involvement of the skin and its tendency to heal with scarring.[1] Oral involvement in BP, although well-recognized, is regarded as uncommon, having been reported in between 11% and 45% of patients.[2] Involvement of other mucous membranes rarely occurs and there are no reports to our knowledge of the ophthalmological findings in BP patients.

Correspondence: Dr V.A.Venning, Department of Dermatology, The Slade Hospital, Oxford OX3 75H, U.K.

In the present study we examined patients with BP and CP with particular reference to involvement of the mucous membranes, including a routine assessment by an ophthalmologist in some patients. Both clinical and direct IF findings are reported.

METHODS

Patients

Bullous pemphigoid. Thirty-six patients presenting to dermatology clinics in the Oxford region were diagnosed as having BP by the presence of non-scarring sub-epidermal blisters of the skin and positive direct IF for IgG or C_3 in the basement membrane zone of uninvolved skin. There were 21 males and 15 females with an average age of disease onset of 70·6 years (range 49–89 years), and with a disease duration of 1 week to 10 years (mean 25 months). At the time of the study eight patients were receiving no treatment; the remainder were taking a variety of disease modifying drugs, usually prednisolone, azathioprine or dapsone, alone or in combination. The majority of patients had mild to moderate disease activity; five had florid active disease, and seven had no lesions when they were studied.

Cicatricial pemphigoid. Fifteen patients were diagnosed as having cicatricial pemphigoid by reason of sub-epidermal blisters affecting predominantly the mucous membranes or because of unequivocal cutaneous scars. They were recruited from departments of dermatology and oral surgery and none was, therefore, known to have a cicatrizing conjunctivitis at presentation. Eleven patients had skin involvement in addition to mucosal lesions, and in six scarring was present. The diagnosis had been supported by positive direct IF consistent with CP in all but three patients. These three had repeatedly negative findings, but their disease was in other respects typical of CP. The group consisted of four males and 11 females with a mean age of onset of 59 years (range 45–78 years) and the duration of disease varying from 3 weeks to 19 years (mean 59 months). Five patients were on no treatment; four were receiving topical steroids only, and five were taking immuno-suppressive treatment. All but three patients had evidence of disease activity at the time of the study.

Controls

The ophthalmological findings in the patients were compared with those in a group of 20 controls (six males, 14 females, mean age 61 years). The controls were selected from patients undergoing ocular surgery for conditions unrelated to conjunctival disease. Any patient with known primary conjunctival disease was excluded.

Clinical Assessment

In all patients a detailed history was taken of symptoms affecting the skin, mouth, pharynx, nose, eyes and perineum, and these sites were also examined for the presence of lesions, usually on several occasions. Twenty-one patients with bullous pemphigoid and 14 with cicatricial pemphigoid were referred for routine assessment by the same ophthalmologist (P.A.F.) who had no prior knowledge of the patients' diagnostic category.

Tissue biopsies

Where consent was obtained, single 3 mm punch biopsies were taken from uninvolved forearm skin and from uninvolved lower lip mucosa for direct IF studies; the pairs of biopsies being

TABLE 1. Clinical and immunofluorescence findings in 36 patients with bullous pemphigoid

Patient no.	Sex	Age at onset (years)	Disease duration	Clinical features							Direct immunofluorescence of uninvolved tissue		
				Eyes Symptoms	Eyes Signs	Skin	Mouth	Nose	Pharynx or larynx	Vulva or urethra	Skin	Mouth	Conjunctiva
1	M	75	1 month	–	fs	+	+	–	–	–	IgG, C_3	IgG, IgA, C_3	IgG, C_3
2	M	74	10 months	R	fs	+	+	R	–	–	C_3	C_3	C_3
3	F	72	20 months	–	–	+	R	–	+	–	IgG, C_3	–	IgG
4	F	49	1 month	+	–	+	+	–	–	+	C_3	C_3	–
5	F	85	6 months	–	fs	+	+	–	–	+	IgG, C_3, C_{1q}	IgG, IgA, C_3	–
6	M	78	< 1 month	+	–	+	+	–	R	–	IgG, C_3	IgG, C_3	–
7	M	58	10 years	R	fs	+	R	+	–	–	–	C_3	IgG, C_3
8	M	54	1 month	+	–	+	–	–	–	–	–	C_3	C_3
9	F	51	4 years	+	symb.	+	R	–	–	–	–	C_3	C_3
10	M	65	3 months	+	–	R	R	R	–	–	–	IgG	–
11	M	76	7 years	+	–	R	R	–	–	–	–	–	IgG, C_3
12	F	82	5 years	+	–	R	R	R	–	–	–	–	IgG
13	F	80	3½ years	+	fs	R	R	–	–	–	–	–	IgG
14	M	75	12 months	+	fs	+	R	–	–	–	–	–	IgG
15	M	70	< 1 month	–	–	+	+	–	–	–	IgG, C_3	–	C_3
16	M	83	14 months	+	–	+	–	–	–	–	IgG	IgG	IgG, C_3
17	M	54	3½ years	–	–	+	–	–	–	–	–	IgG, C_3	–
18	F	83	7 months	–	–	+	–	–	–	–	–	–	–
19	M	70	5 years	–	–	+	–	–	–	–	–	–	C_3
20	M	81	3 years	+	fs	+	–	–	–	–	–	–	ND
21	F	70	3½ years	–	–	+	–	–	+	–	IgG, C_3, C_{1q}	IgG, C_3	ND
22	M	67	14 months	–	ND	+	+	–	–	–	IgG, C_3	IgG, C_3	ND
23	M	72	7 years	–	ND	R	R	–	–	+	–	C_3	ND
24	M	81	2 years	–	ND	+	–	–	–	–	–	IgG, C_3	ND
25	M	62	2 years	–	ND	R	–	R	–	–	–	–	ND
26	M	87	3 months	–	ND	+	–	–	–	–	IgG, IgA, C_3	IgG, C_3	ND
27	M	75	1 month	–	ND	+	–	–	–	–	IgG, C_3, C_{1q}	–	ND
28	F	89	< 1 month	–	ND	+	+	–	–	–	IgG, C_3, C_{1q}	IgG, C_3	ND
29	F	69	9 months	–	ND	+	–	–	–	–	–	–	ND
30	F	69	1 month	–	ND	+	–	–	–	–	IgG, C_3	IgG, C_3	ND
31	M	72	8 years	–	ND	R	–	–	–	–	–	–	ND
32	F	83	3 months	–	ND	+	–	–	–	–	IgG, C_3	IgG, C_3	ND
33	F	71	18 months	–	ND	R	R	–	R	–	–	–	ND
34	M	83	< 1 month	–	ND	+	–	–	–	–	C_3	–	ND
35	F	73	< 1 month	–	ND	+	–	–	–	–	C_3	IgG	ND
36	F	81	3½ years	+	ND	+	–	–	–	+	IgG, C_3	ND	ND
Total				14	8 of 21	36	18	5	4	4	18 of 36	20 of 35	13 of 19

+ = active lesions present at the time of study; R = remission; fs = fine scarring; symb. = symblepharon; ND = not done.

taken at the same clinic visit. Thirty-five patients with BP had biopsies taken from skin and lip; of these, 18 gave a history of recurrent oral ulceration or blisters of whom eight had lesions present at the time of the study. Fourteen of the 15 CP patients consented to biopsies; 13 had a history of oral involvement, of whom seven had lesions present at the time of the study. Biopsies were taken from approximately the same site in all patients and in none was this site lesional or peri-lesional. The lip biopsies were taken from the midline, inside the lower lip.

Nineteen patients with BP, 12 with CP and seven of the controls consented to a biopsy of uninvolved bulbar conjunctiva for IF studies.

All biopsies were embedded in OCT medium and snap frozen in liquid nitrogen. Cryostat sections of the biopsies were examined for the presence of IgA, IgG, IgM and C_3, C_4 and C_{1q}, using a standard direct IF technique.

RESULTS

Bullous pemphigoid

The results are listed in Table 1. Twenty-one of the 36 BP patients (58%) gave a history of involvement of one or more mucous membranes. The mouth was the most commonly affected site with blisters or ulcers affecting the palate, cheeks, lips and tongue in 18 patients. In only two patients were oral lesions the initial manifestation of their disease. In the remainder, these developed either during the acute phase of the bullous skin eruption or subsequently in the course of the disease.

Recurrent crusting and bleeding of the nasal mucosa occurred in five patients; pharyngeal soreness and dysphagia occurred in three patients with haemorrhagic bullae of the hypopharynx seen on indirect laryngoscopy. One patient (Patient 21, Table 1) whose disease had been confined to the skin for 3½ years, developed an upper oesophageal web for which no cause could be found. The mucosal web was friable and biopsy material was, therefore, unobtainable. One male patient had a recurrent sore eroded area surrounding the urethral meatus and three females had vulval ulceration.

Fourteen patients reported ocular symptoms, most commonly consisting of mild grittiness, soreness or dryness of the eyes. One patient had severe photophobia. Of the 21 patients examined by the ophthalmologist, seven had fine scarring of the tarsal conjunctiva, in some accompanied by conjunctival injection or chemosis. Two patients had marked conjunctival injection without fine scars and one patient had a definite broad symblepharon with obliteration of the lower conjunctival fornix (Patient 9, Table 1).

Eleven of the 21 patients with mucosal involvement had lesions confined to a single site (mouth 7, nose 1, pharynx 1, vulva 2) (see Table 1). Involvement of two mucosal sites occurred in 10 patients (mouth and eyes 1, mouth and nose 4, mouth and pharynx 3, mouth and vulva 1, mouth and urethra 1).

Immunofluorescence studies. Linear deposition of IgG and C_3 either alone or in combination, was detected by direct IF in 18 of the 36 skin biopsies (50%) and in 20 of the 35 lip biopsies (57%). One skin biopsy and two lip biopsies also contained IgA (Patients 1, 5 and 26, Table 1). The 20 patients with positive direct IF in the lip biopsy, included seven of eight patients with oral lesions present at the time of the biopsy, four of 10 with a past history of oral lesions and nine of 17 with no history of oral involvement.

The frequency of positive IF in both skin and lip was broadly related to disease activity, being present in both tissues in four of five patients with very active disease, but less frequently in mild

TABLE 2. Clinical and immunofluorescence findings in 15 patients with cicatricial pemphigoid

				Clinical features							Direct immunofluorescence of uninvolved tissue		
Patient no.	Sex	Age at onset (years)	Disease duration	Eyes		Skin	Mouth	Nose	Pharynx and larynx	Vulva and urethra	Skin	Mouth	Conjunctiva
1	F	56	12 months	R	fs	R	+	–	–	R	–	–	C_3
2	F	52	15 months	+	–	Rs	+	R	–	–	IgG, IgM	IgG, C_3	IgG
3	F	62	11 years	+	fs	–	R	–	–	–	–	IgG, C_3	IgG
4	F	50	19 years	+	symb.	Rs	R	R	–	R	–	–	IgG, C_3
5	F	60	5 months	+	marked chemosis	+s	+	+	R	–	IgG, C_3	C_3	IgG, C_3
6	F	80	1 month	+	symb.	+	+	–	–	–	–	IgG	IgA, IgG
7	M	78	1 month	–	–	+	R	–	R	–	IgG, C_3	IgG, C_3	–
8	M	59	5 years	–	fs	+s	–	–	+	–	–	–	–
9	F	52	4 years	R	fs	–	+	+	–	R	–	–	–
10	M	55	2 years 4 months	R	fs	Rs	R	R	R	R	–	–	–
11	F	45	12 years	+	–	–	R	+	R	R	–	–	–
12	F	55	2 years	+	symb.	–	+	+	–	+	–	ND	–
13	M	72	2 years	+	fs	Rs	R	–	R	–	–	–	ND
14	F	50	14 years	–	fs	+	+	–	+	+	–	C_3	ND
15	F	58	< 1 month	–	ND	+	+	+	+	–	C_3, C_{1q}	–	ND
Totals				11	11 of 14	11	14	8	8	7	4	6 of 14	6 of 12

+ active lesions present at the time of study; R = remission; fs = fine scarring; s = scarring cutaneous lesions; symb. = symblepharon; ND = not done.

to moderately active disease (skin positive in 14 of 24 patients, lip positive in 14 of 18 patients) and rarely in remission, (skin positive in 0 of six patients, lip positive in two of eight patients).

Linear deposition of IgG and C_3 at the dermo-epidermal junction, either alone or in combination, was detected on direct IF in 13 of the 19 conjunctival biopsies, including patients with neither ocular symptoms nor signs. The only patient with significant scarring had linear C_3 deposition (Patient 9, Table 1).

Cicatricial pemphigoid

The results are shown in Table 2. All 15 patients had involvement of the mucous membranes and 11 patients also had skin lesions, six with scarring. Blisters or ulcers of the palate, lips, gums or tongue were present in 13 patients and one had desquamative gingivitis. Eight patients had recurrent crusting or bleeding of one or both nostrils and one patient also had adhesions of the nasal mucosa. Eight patients had pharyngeal bullae, six had vulval erosions and two had recurrent dysuria with negative urine cultures. One patient had recurrent perianal ulcers (Patient 9, Table 2). Fourteen patients were examined by the ophthalmologist; 11 reported ocular symptoms and 11 had ocular signs (fine scarring of the tarsal conjunctiva with or without conjunctival injection in seven patients, symblepharon in three and one patient with persistent severe chemosis and ulceration of the tarsal conjunctiva).

Mucosal involvement tended to be widespread in this group, with seven patients having lesions at three or more different sites. Only one patient had lesions confined to a single site, a man who had pharyngeal bullae accompanying scarring skin lesions (Patient 8, Table 2).

Immunofluorescence studies. Three patients had repeatedly negative IF from the time of diagnosis (Patients 8, 9 and 11, Table 2). The remainder had positive direct IF at the basement membrane zone at presentation in either oral mucosa or skin. At the time of the study, linear basement membrane zone IgG or C_3 or both were detectable in four of the 15 skin biopsies and six of the 14 lip biopsies. Of the six patients with positive lip IF, four had oral lesions present. Twelve patients had a conjunctival biopsy and immunoreactants were deposited in the basement membrane zone in six. Of these six patients, three had significant ocular signs, one of whom had IgA in addition to IgG and C_3 in the conjunctival biopsy (Patient 6, Table 2).

Controls

Mild ocular symptoms and signs similar to those found in the BP and CP patients occurred in nine of the 20 controls. None had symblepharon. Seven controls had a conjunctival biopsy and all had negative direct IF.

DISCUSSION

There are no precise diagnostic criteria to distinguish the sub-epidermal blistering diseases bullous and cicatricial pemphigoid. The terms cicatricial or benign mucous membrane pemphigoid are applied both to patients with scarring lesions and to those with disease confined to mucous membranes. However, these diagnostic terms have also been used for patients who have short-lived, generalized non-scarring bullous eruptions of the skin in addition to lesions of the mucous membranes.[3]

The term bullous pemphigoid is generally applied to patients whose disease is non-scarring and chiefly affecting the skin. Previous authors have regarded oral lesions in such patients as uncommon,[4] although Sneddon and Church[5] report an incidence of 45%. Lesions of other mucous membranes are rarely reported. In the present study of 36 patients with bullous pemphigoid we found a high incidence of mucosal involvement (58%). In keeping with the findings of other authors, the mouth was found to be the site most commonly affected (50%). Several of our patients had either nasal, pharyngeal or perineal lesions and it was not uncommon for two mucosal sites to be affected in addition to the skin (28%). In such patients, where both non-scarring skin lesions and mucosal lesions occur, the distinction between bullous and cicatricial pemphigoid must necessarily be arbitrary and based on the extent and severity of involvement at different sites.

Mild ocular symptoms and signs were present in a similar proportion of BP and CP patients and the group of elderly controls. These common ocular findings consisted of fine scarring of the tarsal conjunctiva accompanied by conjunctival injection or chemosis. Scarring of the symblepharon type was unique to the pemphigoid patients and absent in the controls, and was present in one patient with BP and three with CP. This finding has not previously been described in BP to our knowledge and again emphasises the degree of clinical overlap between BP and CP. Only further investigation will determine whether ocular scarring is a true rare complication of BP or whether patients with this sign are best regarded as having CP. Currently available clinical and immunopathological criteria cannot make this distinction. The low incidence of significant ocular scarring amongst the CP patients is a reflection of the method of selection of patients via dermatology and oral surgery departments, thereby excluding patients presenting primarily with a cicatrizing conjunctivitis. The ophthalmological findings in this study will be reported fully elsewhere (Frith *et al.*, manuscript in preparation).

In BP, positive direct IF has been previously described in 89% of oral biopsies (both perilesional and uninvolved tissue) from untreated patients.[6] In the present study which was mainly of treated BP patients, the presence of immuno-reactants in uninvolved oral mucosa was confirmed, although at a lower frequency, and this was shown not to correlate closely with clinical involvement at this site. Although patients with active oral lesions had the highest frequency of positive lip IF (7 of 8), positive results were also obtained in 40% of patients with previously documented oral lesions and in 53% of patients who had never had any involvement of the oral mucosa. As the biopsies from skin and lip were taken at the same clinic visit, these results allow a direct comparison of the immuno-reactants bound at different sites at a given point in time. They show that patients with active generalized pemphigoid are highly likely to have positive IF in both tissues, whereas those with more quiescent disease activity may have positive IF confined to one tissue only. Of the two sites, lip was slightly more frequently positive than skin (57% compared to 50%), although by pooling the results from both tissues, the yield of positive results was increased to 68%. Punch biopsy of the uninvolved lip mucosa is a quick and simple procedure with low morbidity, and suitable for use in routine dermatology out-patient clinics. We suggest that it should be considered in addition to a skin biopsy in order to increase diagnostic yield, particularly in patients with disease that is limited in extent or is intermittent.

The direct IF findings in conjunctiva in BP have not been reported previously, to our knowledge. Linear IgG or C_3, or both, occurred in 13 of 19 biopsies (68%), an incidence which exceeds that in either lip or skin alone. Furthermore, IMF was positive in some patients with neither ocular symptoms nor signs.

Our findings suggest that immunoreactants are widely deposited in BP in both skin and mucosal basement membrane, presumably due to common antigenic determinants in the different sites. However, binding of immunoreactants does not inevitably result in the sequence of events leading to the formation of a clinical lesion. The high frequency of positive IF in both conjunctival and oral mucosa, compared with skin, cannot readily be explained. Possibly binding at mucosal sites is more tenacious, resulting in persistence of immunoreactants even in waning disease. Local factors, such as vascularity, tissue proteases or relative lack of connective tissue support, may enhance immunoreactant deposition and persistence in mucosal sites.

In CP, the deposition of immunoreactants in the basement membrane zone of the skin,[7] oral mucosa[8] and conjunctival mucosa,[9] has been described previously in biopsies from lesional or perilesional tissue. Positive IF has also been reported in the basement membrane zone of uninvolved skin in CP patients, both with and without a history of cutaneous involvement.[10] We found that, as in BP, immunoglobulins or complement or both may occur in uninvolved oral mucosa and conjunctiva as well as in uninvolved skin, whether or not there is a history of clinical involvement in the respective sites. In this respect CP resembles BP in having widespread deposition of immunoreactants, although not closely related to the distribution of the clinical lesions.

The immunoreactants most frequently found in both BP and CP and in all three sites examined were IgG and C_3, whereas IgA occurred only rarely. Leonard *et al.*[11] reported conjunctival IgA in 6 out of 16 CP patients with ocular involvement. In contrast, we found IgA in the conjunctival basement membrane zone of only one CP patient, although this patient was one of the few with unequivocal ocular involvement. It has also been suggested that in the subepidermal blistering diseases, a relationship exists between IgA deposits in skin and clinical involvement of mucous membranes,[12] a finding we are unable to confirm.

The classification of the subepidermal bullous diseases is unsatisfactory. Bean *et al.*[13] have regarded BP and CP as forming a spectrum in respect of their clinical expression. This idea gained credence during the 1970s from the demonstration that positive direct and indirect IF occurs in CP[7–9,13,14] and is indistinguishable from that seen in BP, although occurring less frequently. Furthermore, the electron microscopic and immuno-electron microscopic findings are similar in the two diseases and show that the lamina lucida is the site of bulla formation[15–16] and immunoreactant deposition.[17–19] More recently, Leonard *et al.* [10] have demonstrated clinical and immunopathological similarities between patients with benign mucous membrane pemphigoid and linear IgA disease, and the relationship of this latter entity to the other subepidermal bullous disorders is also uncertain.

The present study shows that many patients regarded as having BP, by the criteria in common usage, prove to have relatively frequent and widespread clinical involvement of the mucosae when this is specifically sought. This observation makes differentiation of this disease from CP, on clinical grounds, difficult or impossible. Furthermore, in both diseases, immunoreactants are deposited in uninvolved skin, oral mucosa and conjunctiva, precluding their differentiation by standard immunopathological investigation. Nevertheless, despite these striking similarities, we are unable to conclude that the diseases are necessarily a single entity. Arguing against this conclusion are differences in their natural history, with CP showing little tendency to remit, compared with BP,[1,3] a finding we confirm. Furthermore, using sera from BP patients it has been shown that the BP antigen is a normal basement membrane zone component that is synthesized by keratinocytes in culture and that it is a protein of 220 kd.[20] The antigen in CP remains unidentified, although it has been suggested that the *in vivo* binding of immunoreactants that occurs does so in a different part of the lamina lucida from that in BP.[21] The final classification of the subepidermal bullous disorders must await positive identification of the antigen or antigens involved.

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Detection of type III collagen in skin fibroblasts from patients with Ehlers-Danlos syndrome type IV by immunofluorescence

ANNE S.TEMPLE, P.HINTON, P.NARCISI AND F.M.POPE

MRC Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ, U.K.

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SUMMARY

Immunofluorescence showed that cultured skin fibroblasts from 15 out of 17 patients with Ehlers-Danlos syndrome type IV retained abnormal amounts of type III collagen within the cytoplasm. This was not shown by fibroblasts from normal subjects or from patients with other inherited connective tissue diseases. The diagnosis of Ehlers-Danlos syndrome type IV may be facilitated by this finding.

Ehlers-Danlos syndrome (EDS) is a heterogeneous inherited connective tissue disorder which combines loose-jointedness and hyper-extensible fragile skin with a tendency to bruising and bleeding.[1] At least nine types of EDS exist which vary in their severity and mode of inheritance, and identifiable molecular defects have been discovered in some of them.[2–4]

Type IV EDS is a very severe form of the disease, usually leading to premature death from the rupture of large or medium sized arteries. Pneumothoraces and intestinal or splenic rupture also occur. Clinically it can be distinguished from other forms of EDS. Affected patients have thin inextensible skin which shows premature ageing, and have large eyes, a pinched nose and thin lips, together with small stature, club-feet and a tendency to form keloid scars.[4–8] The skin and other tissues such as arterial media, pleuro-peritoneal lining and intestinal walls can be shown to be depleted of collagen and specifically lack collagen type III.[5–9] Skin fibroblasts from patients usually show poor secretion of collagen type III into the medium,[5,8,10,11] but occasionally, other patients with the typical clinical phenotype of EDS IV show normal synthesis of type III procollagen.[9,12] Immunofluorescent staining with anti-type III procollagen antibodies has been shown in fibroblasts of one EDS IV patient by Byers *et al.*[8] but other studies have failed to show staining.[8,10,13] A proteinase sensitive procollagen with an unusual peptide map has also been described.[11] In our laboratory, fibroblasts from most EDS IV patients secrete little or no type III collagen, but commonly retain abnormal protein intracellularly. Other variants are poor secretors, but do not retain protein intracellularly.

Correspondence: Dr A.Temple.

Diagnosis by biochemical and molecular methods is labour intensive, expensive and is only available in a few specialized laboratories. Immunofluorescence would provide a fast, cheap and easier method of diagnosis which is available in many hospital laboratories. In the present study we have examined fibroblasts from authentic EDS IV patients in many of whom the diagnosis had been confirmed biochemically.

METHODS

Ehlers-Danlos patients

The 17 patients, skin biopsies from whom were included in the study, had all originally been referred to one of us for confirmation of the clinical diagnosis and for biochemical investigation

TABLE 1. Details of patients with Ehlers-Danlos syndrome included in the study

Case no.	Sex	Age at biopsy	Clinical features	Type III collagen as % of type I + type III**	Complications
1‡	F	52	Typical facies;* club feet	11	Arterial rupture
2‡	F	47	Typical facies; club feet	12	Died, arterial rupture
3	F	45	Typical facies	0·4	Died, arterial rupture and venous tear
4	M	42	Varicose veins; thin skin	3	Pneumothoraces
5	F	32	Typical facies	2	Popliteal artery aneurysm
6	F	31	Typical facies	2	Died, ruptured spleen; previous hepatic rupture
7	M	30	Typical facies	1	Died, arterial rupture
8	F	28	Typical facies; club feet	1	Several arterial ruptures
9	F	25	Typical facies	<2	Died, aortic rupture
10	F	22	Typical facies	2	Colonic rupture
11‡	M	21	Milder disease	13	Died, ruptured spleen, previous axillary aneurysm
12	F	17	Typical facies	14	None
13	M	13	Thin skin; bruising	1	Bruising
14	F	12	Typical facies	6	Internal carotid artery dissection
15	F	8	Thin skin	3	Extensive bruising; normal haematology
16	M	3	Resembled EDS II, but mother had typical facies and arterial rupture	3	Bruising
17	M	3	Typical facies	2	Bruising

*Typical facies: delicate nose, large eyes, thin lips.

**Normal range 13–16%

‡Patients 1 and 2 had linkage of disease to type III gene; patient 11 may have had structural protein mutation.

(Table 1). All had had one or more of the typical clinical features of EDS IV listed above, and nine had suffered major arterial catastrophies which had been fatal in five of them. The majority were type III collagen deficient as measured by polyacrylamide gel electrophoresis of radiolabelled pepsinized collagens produced in culture. Exceptions were two patients from a large autosomal dominant pedigree who showed minimal type III collagen deficiency, but who showed tight linkage of the disease to a type III collagen restriction fragment length polymorphism[14] and one other patient with a milder clinical history.

Anti-collagen antibodies

Types I and III collagen were prepared from human placentae by the method of Rhodes and Miller[15] and estimated to be greater than 95% pure by polyacrylamide gel electrophoresis.

Goats were injected intramuscularly and sub-cutaneously with 1 mg type I or type III collagen in 2 ml Freunds complete adjuvant (Difco Laboratories, East Molesey, U.K.), and bled 3–5 weeks later. Subsequent injections were 2–3 months apart, and the animals were exsanguinated after five injections.

Fibroblast cultures

Fibroblasts from human skin biopsies were grown to confluence in plastic petri dishes in Dulbecco's minimal essential medium (DMEM) supplemented with 10% foetal calf serum, 2 mM glutamine, 40 μg/ml ascorbic acid (Analar, BDH Chemicals Ltd., Poole, U.K.) and containing 100 IU/ml streptomycin and 100 IU/ml penicillin. (All tissue culture reagents were obtained from Flow Labs Ltd., Irvine, Scotland). Passage number varied between p4 and p17. Cell lines from normals or from other connective tissue diseases were included in all experiments and cultures were 'blind-labelled' before use. After trypsinization 2×10^4 cells in 0·4 ml were aliquoted into 1 cm^2 chambers in 4808 Lab-Tek tissue culture chamber/slides (Miles Labs Ltd., Stoke Poges, Bucks, U.K.). Cell density was checked visually each day and immunofluorescence of collagen was performed when cell numbers were moderate and again near confluence. The medium was changed every 2 days.

TABLE 2. Reactivity of goat anti-human collagen antisera in an enzyme-linked immunoabsorbent assay

Antigen	Concentration	Anti-type I collagen	Anti-type III collagen
Type I collagen	2 μg/ml	1:100,000	1:600
Type III collagen	2 μg/ml	1:600	1:20,000
Type IV collagen	5 μg/ml	1:5000	1:6,400
Type V collagen	5 μg/ml	1:150	1:60
Human serum	50 μg/ml	<1:10	<1:10

Antisera were obtained after three injections of collagen in Freund's complete adjuvant.

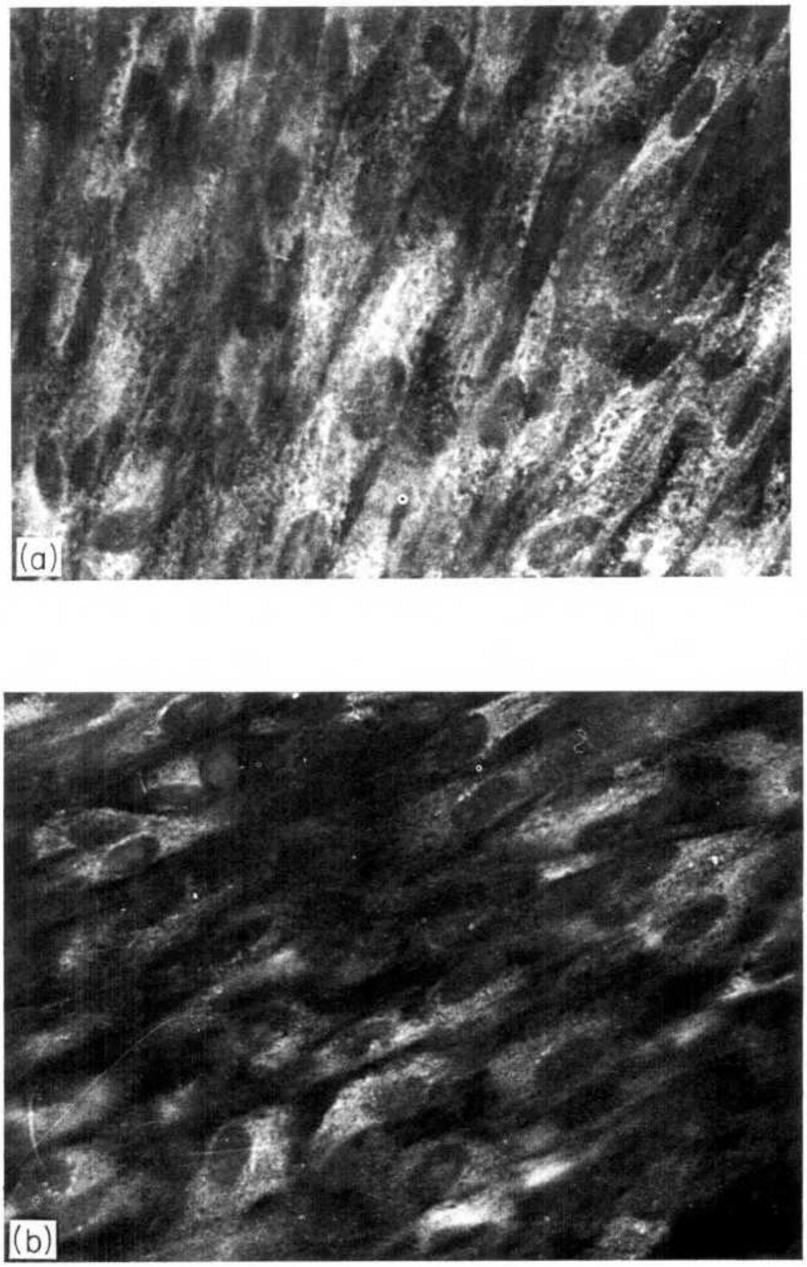

FIGURE 1. Immunofluorescence of types I and III collagen in human fibroblasts. (a) Cells from an EDS IV patient with anti-type 1 collagen antibody; (b) normal cells with anti-type III collagen antibody (original × 530).

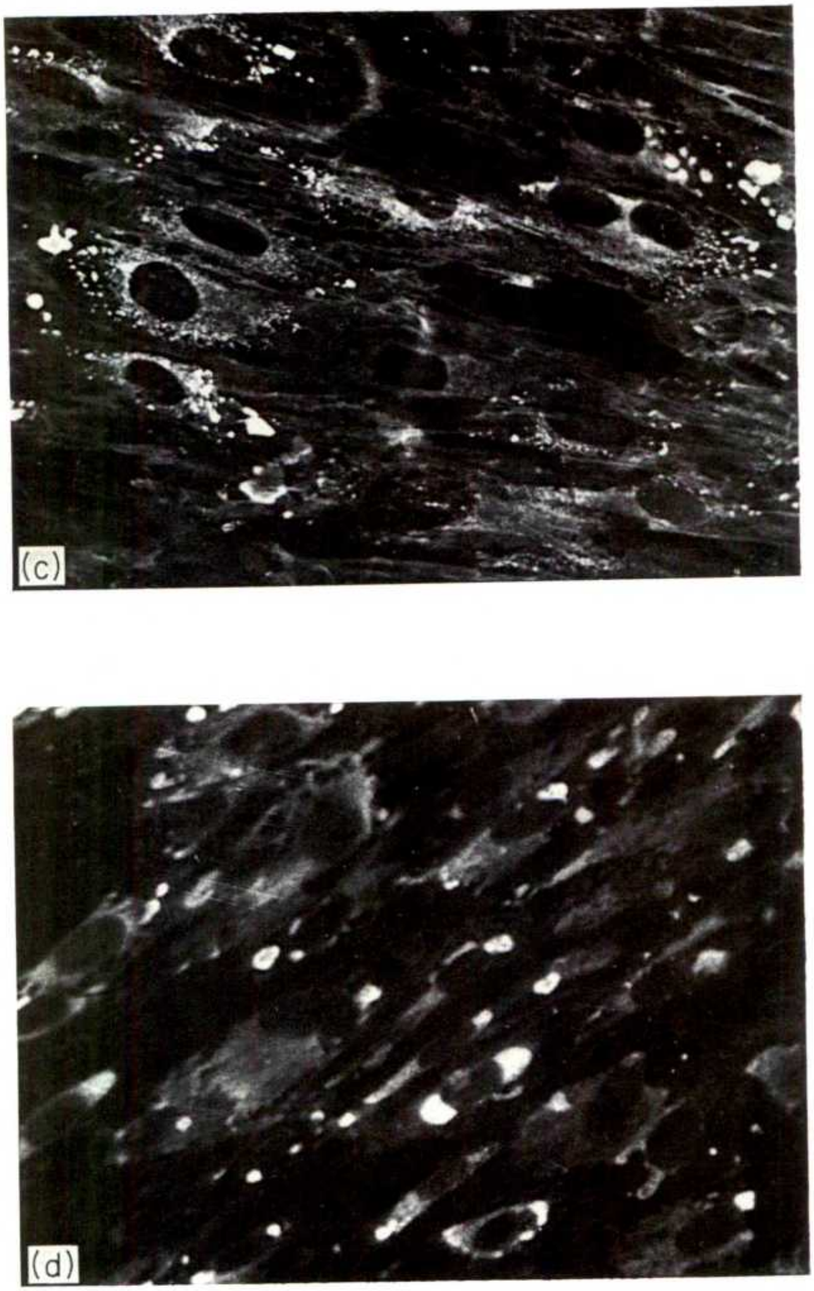

FIGURE 1. Immunofluorescence of types I and III collagen in human fibroblasts. (c) and (d) Cells from two EDS IV patients with anti-type III collagen antibody (original × 530).

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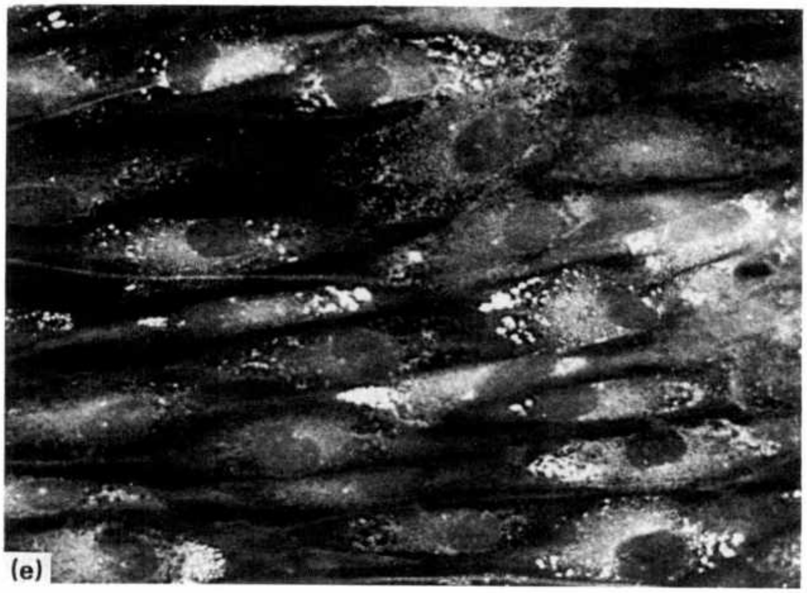

FIGURE 1. Immunofluorescence of types I and III collagen in human fibroblasts. (e) Normal cells after treatment with 10 μM monensin, with anti-type III collagen antibody (original × 530).

Immunofluorescence

All washes and serum dilutions were done in DMEM unless otherwise stated and the slide chambers were never allowed to dry. The culture slides were washed three times at 37°C, fixed in methanol at 4°C for 2 min and washed twice before incubation for 10 min at room temperature in medium containing 1% bovine serum albumin (BSA) (Armour Pharmaceutical Co. Ltd., Eastbourne, U.K.). The BSA was carefully removed using a pipette and 0·1 ml of 1:20 goat antiserum or normal goat serum in 1% BSA was added. This dilution was found to be optimal because normal goat serum gave no background staining at 1:20 dilution. The slide chambers were incubated at room temperature for 20 min on a platform rocking at 10 rpm. After three further washes a 1:20 dilution of FITC-conjugated rabbit anti-goat immunoglobulin was added (Dako Ltd., High Wycombe, U.K.) and re-incubated as above. After two quick washes the plastic sides of the chamber were snapped off, the flexible mounting was removed and the slides washed twice in 200 ml of phosphate buffered saline (PBS). The slides were then mounted in 50% glycerol in PBS and examined under a Zeiss photomicroscope III using an HBPO 50 Watt mercury lamp.

Collagenase treatment

A highly purified bacterial collagenase (Advance Biofactures Corp., New York, U.S.A.) was dissolved in water and diluted in serum free DMEM containing 5 mM N-ethylmaleimide and 50μg/ml phenylmethyl-sulfonyl fluoride (Sigma Chemical Co. Ltd., Poole, U.K.) Methanol-fixed fibroblasts were washed three times before the addition of collagenase, and incubated in 95% humidity at 37°C for 4 h or overnight.

Monensin treatment

Fibroblast cultures in chamber slides were incubated for 24 h in complete Dulbecco's medium containing 10^{-5}M monensin (Sigma Chemical Co. Ltd., Poole, U.K.) before being used for immunofluorescence.

ELISA assay

The antisera used for immunofluorescence were assayed in micro-ELISA plates (Don Whitley Scientific, Shipley, U.K.) coated overnight at 4°C with various collagens or human serum and then reacted with serial double dilutions of goat anti-collagen types I and III antisera. This was followed by treatment with peroxidase-conjugated anti-goat immunoglobulins (Dako Ltd, High Wycombe, U.K.) diluted 1:1000. The peroxidase substrate used was hydrogen peroxide and o-phenylenediamine (Sigma Chemical Co. Ltd., Poole, U.K.).

RESULTS

Hyperimmune goat antisera against type I and type III collagens were compared using an ELISA technique (Table 2). This is an extremely sensitive method for detecting antigen-antibody reactions, so is suitable for studying the specificity of antisera. Wells were coated with 2 ng of the immunizing collagen reacted with antiserum at a dilution of 1:200. Goat anti-type I collagen antiserum reacted strongly to type I collagen, much more weakly to types III and IV, and very weakly to type V collagen. However, type IV collagen is not synthesized by dermal fibroblasts. Goat anti-type III collagen antiserum reacted strongly to type III collagen and very weakly to types I, IV and V. ELISA plates coated with high concentrations of human serum gave no reaction with either antiserum (Table 2). Western blots of various collagens prepared in our laboratory were treated with antisera to type I and type III. Types I, III and V could be visualized, but no other reaction was demonstrated (data not shown) so it is unlikely that a non-collagenous contaminant contributed to these reactions.

Fibroblast lines were tested for type I and type III collagens both intracellularly and in the surrounding matrix. Cells from normal subjects and patients with other connective tissue diseases were studied in parallel. Intracellular type I collagen was localized mainly in the perinuclear region as fine granular staining (Fig 1a) and could often be demonstrated in the extracellular matrix. Very occasional cells with larger granules occurred but were found equally frequently in controls and patients. Immunofluorescent staining with anti-type III collagen antisera was much weaker than with anti-type I and in many control cultures the staining was barely detectable (Fig 1b) presumably because there is less type III in the cells than type I. Matrix staining was very rarely observed. In contrast, fibroblasts from EDS IV patients consistently showed large granules containing type III collagen which varied greatly in size but always stained extremely brightly (Fig 1c and d). These granules occurred in fibroblasts from 16 of 17 EDS IV patients, but were not seen in eight control lines nor in eight of nine lines from other inherited defects of connective tissue. The latter group consisted of two patients with cerebral aneurysms, two with different types of osteogenesis imperfecta, three patients with EDS I and one with pseudoxanthoma elasticum. Fibroblasts from one aneurysm patient without EDS IV did show occasional bright granules, as did the clinically normal sister of an EDS IV patient.

Treatment with highly purified bacterial collagenase for 4 h at 37°C of fibroblasts from one control and one EDS IV patient abolished type III collagen staining in both cultures, and also all intracellular staining of type I. Some type I in the matrix was still present.

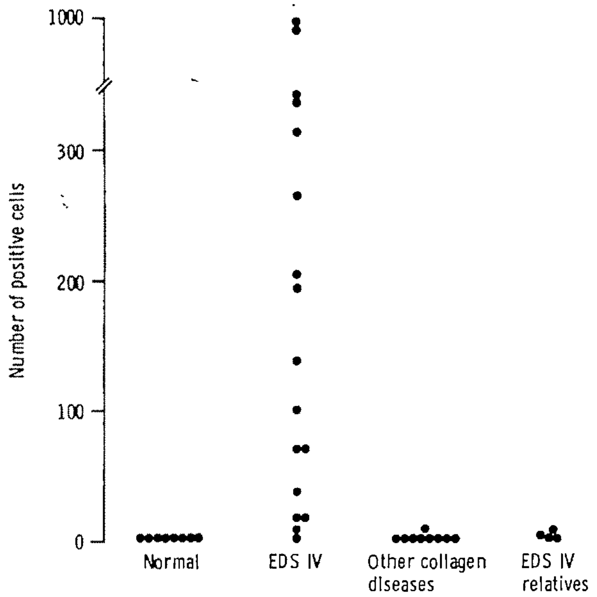

FIGURE 2. Numbers of cells containing large granules staining for type III collagen per 5 mm^2 of sub-confluent fibroblasts from patients with EDS IV and their relatives, patients with other collagen diseases and normal controls. Each point represents a fibroblast culture from one subject.

Immunofluorescent staining of fibronectin using goat anti-human fibronectin antibody (Sigma Chemical Co. Ltd., Poole, U.K.) was unaffected by treatment with 200 U/ml collagenase for 18 h at 37°C. Fibroblasts showed intense staining of intracellular and extracellular fibronectin, showing that the collagenase had no other proteolytic activity.

Fibroblasts from one normal and one EDS IV patient were incubated overnight in monensin, an inhibitor of procollagen secretion.[16] If the immunofluorescent staining pattern of EDS IV cells was caused by the retention of type III collagen, then normal fibroblasts treated with monensin should show a similar pattern. Figure 1e demonstrates this. The EDS IV fibroblasts also showed increased staining. A similar pattern was obtained for collagen type I, although the size of the granules was smaller.

To measure the proportion of cells with abnormal granules, sub-confluent monolayers were scanned several times across the 1cm growth area using a × 25 objective, covering 5 mm^2 per scan. The average number of granule-containing cells per scan was counted. Cultures from two separate passages of each cell line were tested and the highest result is presented (Fig. 2). Parallel studies with anti-type I collagen antibody showed only occasional cells containing abnormal granules, and no difference between disease and control cultures was observed.

DISCUSSION

Type III collagen deficiency is characteristic of EDS IV, but its measurement is labour intensive and requires specialised skills. Our experiments suggest that immunofluorescent staining using anti-human type III collagen antibodies is a simple, rapid test for the EDS IV phenotype. We successfully identified 15 of 17 EDS IV patients. Fourteen of the 17 could be identified by the biochemical analysis of radiolabelled collagen synthesized by fibroblasts, the other three on clinical features. One patient not identified as EDS IV by immunofluorescence did not have abnormal type III collagen using biochemical techniques either, and two patients whose fibroblasts did not appear to retain type III collagen by biochemical methods stained intensely with anti-type III collagen antibodies. Possibly the type III collagen in these cells is abnormally sensitive to degradation during extraction procedures. The abnormal staining pattern of type III collagen detected in one cerebral aneurysm patient and in a sibling of an EDS IV patient suggests that a type III collagen mutation may have occurred in these individuals.

The morphology of immunofluorescent staining varied from large granules to small beads, but was easily recognized by its abnormal intensity. Fibroblasts with large inclusions may have the distension of the rough endoplasmic reticulum described by Holbrook and Byers.[17] Staining with anti-type I collagen antiserum was similar in EDS IV and normal fibroblasts, so can be used as a control for type III collagen.

Collagenase abolished the intracellular staining for both type I and type III collagen, but had no effect on fibronectin, suggesting that the immunofluorescent staining was directed against the helical part of the collagen molecule. Monensin, an ionophore which inhibits procollagen secretion[16] caused fibroblasts to retain both type I and type III collagen, as shown by immunofluorescence. The pattern was similar to that given by type III in fibroblasts from EDS IV so it is probable that the abnormal type III collagen produced by EDS IV fibroblasts cannot be secreted. So far, attempts to determine the ultrastructural distribution of type III collagen have been unsuccessful. All the fixatives used appear to mask the epitopes recognized by the antisera.

Immunofluorescence is more widely used in diagnostic laboratories than the specialized biochemical techniques needed for collagen analysis, and the high proportion of EDS IV patients that were successfully identified in the present study suggests that immunofluorescent staining for type III collagen may serve as a fast and reliable method for diagnosis of the disease.

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The presence of neutrophils in human cutaneous ultraviolet-B inflammation

J.L.M.HAWK, G.M.MURPHY AND C.A.HOLDEN

Department of Photobiology, Institute of Dermatology, St. Thomas' Hospital, London SE1 7EH, U.K.

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SUMMARY

We report the first observation of neutrophils in the dermal inflammatory infiltrate in human skin soon after moderate ultraviolet-B (UVB) irradiation. Thus human UVB-induced cutaneous inflammation, in this as in other respects, appears to resemble other human acute inflammatory responses to injury.

Neutrophils and mononuclear leukocytes are found in the dermis during the acute inflammatory reaction after injury in animals and man, and are very probably causally associated with development and progression of this response.[1,2] Neutrophils are most prominent early in the reaction, but have not been noted at that stage of acute cutaneous UVB inflammation in man[3–7] except after very high doses, sufficient to induce widespread epidermal necrosis.[8] However, they have been found early in this reaction in guinea pigs,[9,10] and their absence from the early UVB reaction in man has been considered an unusual feature of the human reaction. For this reason we have examined tissue sections in detail for the presence of neutrophils in the human dermal inflammatory cellular infiltrate during the first 48 h after moderate UVB irradiation.

METHODS

Eight normal Caucasian volunteers (five males, three females; mean age 40 years range 26–60 years) participated in the study. A Westinghouse FS20 lamp was used to irradiate nine separate $2{\cdot}7\ \mathrm{cm}^2$ skin sites on the buttocks of each subject with four times the pre-determined minimal dose of predominantly UVB radiation required to produce mild confluent erythema of the irradiation site with clearly defined boundaries (minimal erythema dose; MED).

Punch biopsies, 3 mm in diameter and at least 2 cm apart, were obtained under lignocaine local anaesthesia without adrenalin from a nearby unirradiated control site and from the centre

Correspondence: Dr J.L.M.Hawk.

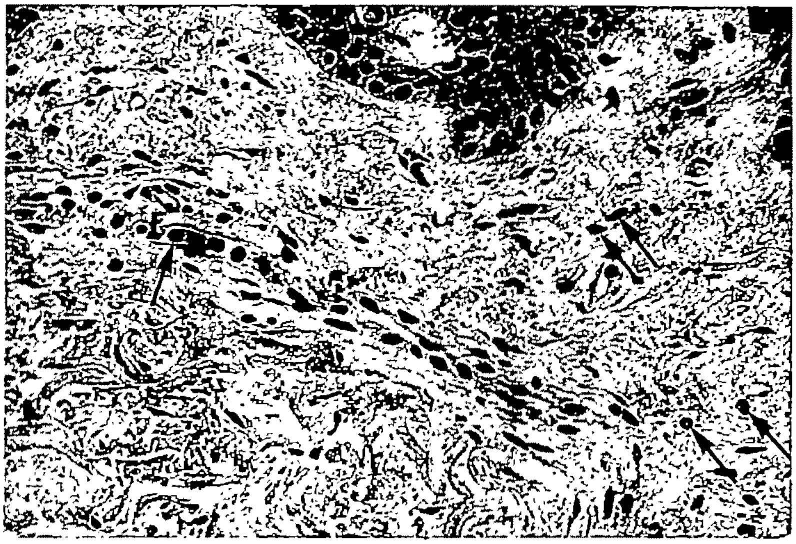

FIGURE 1. Intradermal neutrophils (arrows) 8 h after UVB irradiation. (Original × 40).

of each irradiated area immediately (eight subjects) and at 1 (7), 4 (8), 6 (7), 8 (7), 11 (7), 14 (6), 21 (7), 30 (6) and 48 h (2) after irradiation.

The biopsies were processed routinely, stained with haematoxylin and eosin or chloroacetate esterase and the cells in the dermal infiltrate identified and counted in five high power fields (HPF) in one section of each biopsy. The neutrophil, but not the mononuclear cell, distribution was skewed such that logarithmic transformation of the former data was necessary, each zero

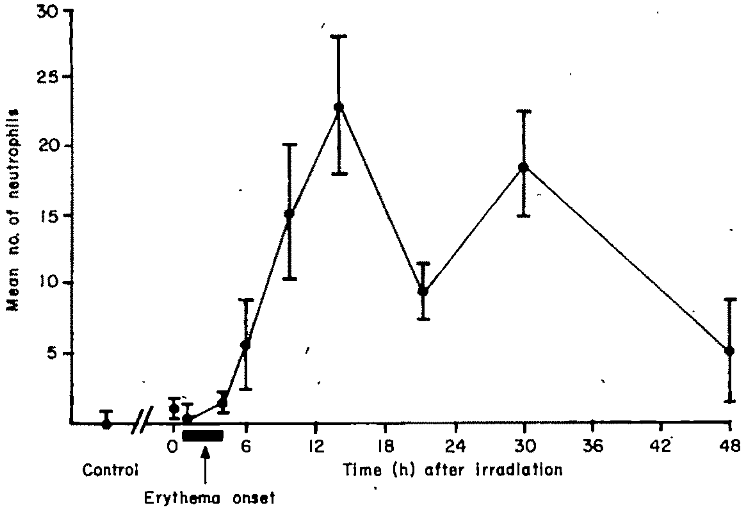

FIGURE 2. Numbers of neutrophils in the dermis after UVB irradiation. Values are means per 5 HPF ± SEM.

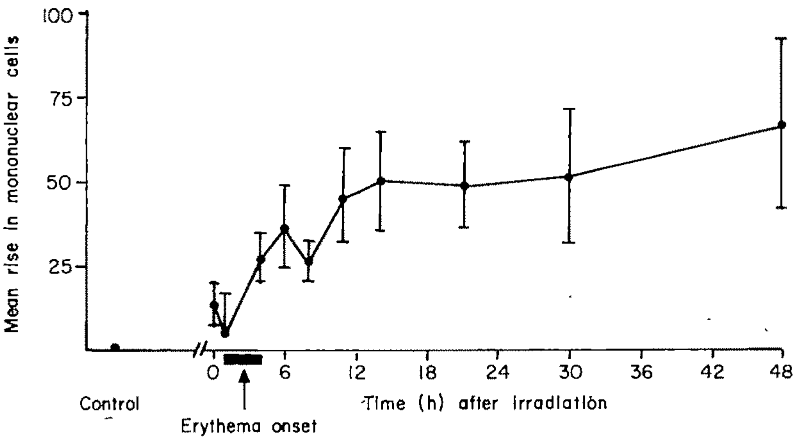

FIGURE 3. Numbers of mononuclear cells after UVB irradiation. Values are mean increase above baseline per 5 HPF ± SEM. Baseline = unirradiated control site; mean cell number 50 cells per 5 HPF.

value being approximated to 1 to enable this. Statistical analysis was carried out using two-way analysis of variance.

RESULTS

Erythema appeared in all subjects ½–4 h after irradiation and was maximal at 24–48 h, the exposed skin being deeply red, just palpable and not blistered. Histologically, the epidermis initially appeared normal, with development of spongiosis between 14 and 21 h after irradiation and mild vesiculation by 24 h. Sunburn cells were present from 1 h after irradiation and reached a maximum of 140 per 5 HPF by 30 h. There was no tissue necrosis.

Dermal neutrophils (Fig. 1) appeared immediately after irradiation. Initially intravascular and sparse they later became perivascular and increased in number from the onset of erythema to a maximum 25 per 5 HPF at 14 h, dwindling thereafter up to 48 h (Fig. 2); a total of two dermal neutrophils only was seen in the eight control biopsies. Dermal mononuclear cells, present before irradiation at a baseline level of approximately 50 per 5 HPF, increased gradually in number after irradiation to a broad plateau of approximately 50 cells per 5 HPF from 11–21 h and then much more slowly up to 30 h (Fig. 3). The increase in both neutrophil and mononuclear cell numbers following irradiation was statistically significant compared with unirradiated control sites ($P < 0{\cdot}01$).

DISCUSSION

We have observed for the first time, the immediate appearance of neutrophils in the human cutaneous reaction to moderate UVB irradiation, with an increase in the number up to 14 h after irradiation and progressive dwindling thereafter. The failure of previous workers[3–7] to make this observation can probably be attributed to their failure to obtain biopsy material at times of significant neutrophil infiltration.

Acute inflammation is the early reaction of mammalian tissue to the effects of an injurious agent and is directed at repair of the damage.[1,2] There is initial rapid local vasodilatation and increase in vascular permeability, adjacent intravascular neutrophil sludging, perhaps from increased vessel wall stickiness, and passage of these cells into surrounding tissue where they ingest foreign material and attract others of their kind by degranulation and release of inflammatory mediators. Mononuclear cells, also attracted by neutrophil breakdown products, are apparently similar to, but less specialized than, neutrophils, and during their more persistent presence at the injury site are probably associated with later modulation of the inflammatory process.

The present discovery of dermal neutrophil accumulation at the site of cutaneous UVB irradiation damage in humans, although apparently of more gradual onset and less marked than in other forms of acute inflammation,[1,2] strongly suggests that human sunburn is a similar acute inflammatory response, mediated at least in part by neutrophil activity. Such a contention is supported by the presence of neutrophils, albeit sparse, in acute UVA-induced inflammation in humans[11] and by the reduced sunburn response in leukopenic guinea pigs.[10] The quest for exact determination of the cutaneous molecular sites of UV absorption and damage leading to the human sunburn inflammatory response should now be stimulated anew.

ACKNOWLEDGMENTS

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The use of chemically split tissue in the detection of circulating anti-basement membrane zone antibodies in bullous pemphigoid and cicatricial pemphigoid

SUSAN E. KELLY AND FENELLA WOJNAROWSKA*

Departments of Dermatology, Royal Berkshire Hospital, Reading and * Slade Hospital, Oxford, UK.

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SUMMARY

Human skin and mucous membranes were used to detect circulating auto-antibodies by indirect immunofluorescence in 20 patients with bullous pemphigoid and eight with cicatricial pemphigoid. The tissue substrate was used intact and after chemical separation through the basement membrane zone (BMZ) by incubation with 1 M NaCl. Chemically split skin and oral mucosa provided a more sensitive assay for demonstrating circulating anti BMZ antibodies. Use of a battery of substrates increased the number of positives in bullous pemphigoid from 30% detected on monkey oesophagus to 100% (tissue battery). In cicatricial pemphigoid there was an increase in the proportion of positive sera from 13% (monkey oesophagus) to 88% (tissue battery). In addition, a different class of antibody was frequently detected on split tissue substrate suggesting that new antigens are exposed by this procedure.

Bullous pemphigoid (BP) and cicatricial pemphigoid (CP) are chronic blistering conditions manifested by subepidermal bullae. Circulating anti-basement membrane zone (BMZ) antibodies may be detected in patients' serum in both conditions although they are less frequently found in CP. This may be due to insufficiently sensitive detection techniques.[1] The technique most frequently employed is indirect immunofluorescence. The immunoglobulins are directed against the bullous pemphigoid antigen (BPA), a macromolecule which may be present within the lamina lucida of the BMZ and also in association with hemidesmosomes of basal epidermal cells.[2,3] BPA is synthesized by the basal keratinocyte and *in vivo* there exists a large intracellular pool of BPA.[4,5] BPA is therefore a normal surface component of the BMZ of skin and other squamous epithelia.

Various methods of dermal-epidermal separation have been described.[6] Following chemical

Correspondence: Dr S.E. Kelly, Department of Dermatology, Royal Berkshire Hospital, London Road, Reading, Berkshire RG1 5AN, U.K.

splitting of skin through the lamina lucida by incubation with 1 M NaCl, BPA is known to adhere closely to the epidermis.[7]

We have investigated the use of human skin and mucous membranes as substrate in an indirect immunofluorescent technique. In addition to intact tissue we used tissue chemically separated through the lamina lucida.

METHODS

Patients

Twenty patients with BP and eight with CP were included in the study. Diagnosis was made on clinical and histological grounds. Brief clinical details of the patients are listed in Table 1 and full

TABLE 1. Details of patients studied

Patient no.	Sex	Age (years)	Disease duration	Clinical evidence of mucosal involvement	Direct IF of mucous membranes
Bullous pemphigoid					
1	F	82	5 years	–	n.d.
2	M	75	5 months	–	negative
3	M	54	3 years	–	IgG,C_3
4	M	83	14 months	–	IgG
5	F	56	5 years	–	n.d.
6	F	83	7 months	–	negative
7	M	81	2 years	–	IgG,C_3
8	F	70	3½ years	–	negative
9	F	80	3½ years	+	negative
10	M	67	14 months	+	IgG.C_3
11	M	74	10 months	+	C_3
12	M	75	1 year	+	negative
13	M	58	10 years	+	C_3
14	F	60	3 years	+	n.d.
15	M	81	3 years	+	IgG
16	F	57	4 years	+	C_3
17	M	72	6 years	+	negative
18	F	71	18 months	+	negative
19	M	65	3 months	+	IgG
20	M	76	6 years	+	negative
Cicatricial pemphigoid					
1	F	52	4 years	+	negative
2	F	50	14 years	+	C_3
3	F	45	12 years	+	negative
4	F	52	15 months	+	IgG,C_3
5	M	72	2 years	+	negative
6	F	62	11 years	+	IgG,C_3
7	M	55	2 years	+	negative
8	F	56	1 year	+	negative

n.d. = not done.

details are published elsewhere.[8] Serum samples were obtained during periods of active disease, and stored at −20°C. Sera from 10 healthy blood donors with no evidence of skin disease were used as controls.

The BP group comprised eight females (age range 51–82 years) and 12 males (age range 54–83 years). Duration of disease ranged from 3 months to 12 years with clinical evidence of mucosal involvement in 12 patients (60%).

The CP group comprised six females (age range 45–62 years) and two males (aged 55 and 72 years respectively). Disease duration ranged from 1 to 14 years. All patients, by definition, had evidence of mucosal involvement.

Tissue specimens

Normal human skin was obtained by biopsy from the flexor aspect of the forearms of 4 volunteers with no evidence of blistering or of other skin disease. Oral mucosa was obtained from 4 oral surgical patients, vaginal mucosa from a vaginal hysterectomy specimen and conjunctiva from an eyelid repair procedure. None of the specimens came from patients with any evidence of skin diseases.

Each tissue specimen was divided, half being mounted in Ames OCT compound (Ames Co), snap frozen in liquid nitrogen at −70°C and stored at −70°C. The remainder was stripped of any subcutaneous fat and treated with 1 M NaCl, using an adaptation of the method of Scaletta *et al.*[9] Briefly, each tissue sample was immersed in 1 M NaCl for 48–72 h at room temperature until the epidermis could easily be pulled away from the dermis using tissue forceps. The split tissue was snap frozen and stored in liquid nitrogen at −70°C. Cryostat sections (6 μm) were prepared for use as substrate for indirect immunofluorescence, Cryostat sections of monkey oesophagus were also used as substrate for indirect immunofluorescence.

Immunofluorescence

A standard indirect immunofluorescence technique was used. Sera diluted 1:10 in PBS were incubated with sections of substrate for 30 min at 37°C. The sections were then washed in PBS pH 7·4 for 30 min and incubated with fluorescein labelled rabbit anti-human IgG, IgM or IgA (Dako, U.K. F:P ratio 2:3 M). In addition, patients' sera were tested for complement fixation by incubation of substrate sequentially with patient serum, normal human serum diluted 1:5 with complement diluting buffer (a source of complement) and fluorescein labelled rabbit anti-human anti C_3 (Dako).[11]

Type IV collagen is a component of the lamina densa and we used an indirect immunofluorescence technique with antibody to type IV collagen to confirm the site of separation in the samples of skin, oral mucosa, vaginal mucosa and conjunctiva (Fig. 1).

All sections were then examined under ultraviolet illumination with an Olympus microscope.

RESULTS

Intact tissue

Results of indirect immunofluorescence (IF) are summarized in Tables 2 and 3. Human tissue proved to be more sensitive than monkey oesophagus for the detection of circulating anti BMZ antibodies, with 14 of 20 BP sera positive on human skin (Fig. 2) compared with 6 of 20 on monkey oesophagus. On human oral mucosa 12 of 20 BP sera were positive. Human vagina was a less good substrate with only 10 of 20 BP sera positive. Human conjunctiva was technically an

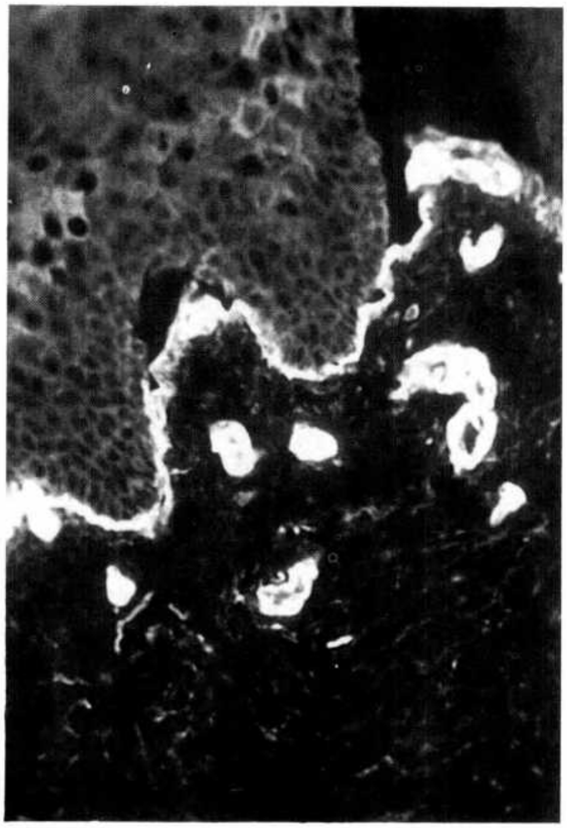

FIGURE 1. Indirect IF. Type IV collagen staining on base of split in split vaginal mucosa (original × 180).

unsuitable substrate which proved difficult to work with but of 14 BP sera tested, 10 were positive (70%) (Fig. 3). No serum was positive on conjunctiva only. Each of the sera was positive on at least one of the human tissues. IgG was the most frequently detected anti-BMZ antibody but IgM and IgA were also detected. Complement fixation as measured by the indirect C_3 assay could be demonstrated with only a proportion of these antibodies.

One of 8 CP sera was positive on monkey oesophagus, 4 of 8 were positive on human skin, 5 of 8 on oral mucosa and 2 of 8 on vaginal mucosa. Three of four CP sera tested on conjunctiva were positive. Seven of eight CP sera (88%) were positive on at least one human tissue substrate.

Split tissue

Compared with intact skin, mucous membrane or monkey oesophagus, separated human skin was the most sensitive substrate for BP and oral mucosa the most sensitive for CP (Tables 2 and 3). Using separated human skin anti-BMZ IgG was detected in 18 of 20 (90%) BP sera. Using separated oral mucosa anti-BMZ IgG was detected in four of eight (50%) CP sera. IgM and IgA were detected but less frequently than IgG.

TABLE 2. Indirect IF on different substrates using BP sera

Patient no.	Monkey oesophagus	Human skin	Split skin	Oral mucosa	Split oral mucosa	Vaginal mucosa	Split vaginal mucosa	Conjunctiva
1	—	—	IgG roof	IgG	IgG,C_3 roof	IgG	IgG roof	IgG
2	—	IgM, C_3	IgG roof	C_3	IgG,C_3 roof	IgG	IgG roof	IgG
3	—	IgG, IgM	IgG roof, IgM base	IgG	—	—	C_3 base	n.d.
4	—	IgM	IgG roof	—	IgG roof	—	IgG roof	IgG
5	—	—	IgG roof	—	IgM roof	—	—	IgG
6	IgG	IgG	IgG comb.	IgG,C_3	IgG roof	IgG,C_3	IgG roof	—
7	—	—	IgG base	—	—	—	IgG, IgM, C_3 roof	IgG
8	—	IgG, C_3	C_3 comb.	C_3	IgG roof	—	IgG,C_3 roof	IgG
9	IgG	IgG, IgA	IgG roof	—	IgG roof	IgG	IgG,IgM roof	IgG
10	—	C_3	IgG comb.	IgG, C_3	IgG roof	IgG, C_3	IgG, IgM C_3 roof	IgG
11	IgG	IgG	IgG, C_3 roof	IgG	IgG, C_3 roof	IgG, C_3	IgG roof	IgG
12	—	IgG	IgG, C_3 roof	—	IgG roof	—	—	IgG
13	IgG	—	C_3 base IgM roof	—	IgG roof	IgG	—	—
14	—	C_3	—	C_3	—	—	—	—
15	—	IgG	IgG comb.	—	—	—	—	—
16	IgG	IgG	IgG comb.	IgG	IgG base	IgG, C_3	IgG roof	IgG
17	IgG	IgG, IgA	IgG comb.	IgG, C_3	IgG roof	IgG, C_3	—	n.d.
18	—	—	IgG base	IgA, C_3	—	—	—	n.d.
19	—	IgG, C_3	IgG comb.	IgA, C_3	IgG roof	IgG,IgA	IgG, C_3 roof	—
20	—	—	IgG comb.	IgG	—	IgG	IgG roof	n.d.

Staining patterns: Roof = roof of blister (epidermal).
Base = floor of blister (dermal).
Comb. = roof and floor of blister (epidermal and dermal).
n.d. = not done.

TABLE 3. Indirect IF on different substrates using CP sera

Patient no.	Monkey oesophagus	Human skin	Split skin	Oral musoca	Split oral mucosa	Vaginal mucosa	Split vaginal mucosa	Conjunctiva
1	—	IgM	IgG, C_3 roof	IgM, C_3	—	—	—	n.d.
2	IgG	IgG	IgG comb.	IgG, C_3	IgG roof	IgG, C_3	IgG,IgM,C^3	IgG
3	—	—	—	—	—	—	—	n.d.
4	—	IgG, C_3	—	IgG, C_3	IgG roof	IgG	—	IgG
5	—	IgM, C_3	—	C_3	—	—	—	n.d.
6	—	—	IgG base	—	—	—	—	n.d.
7	—	—	C_3 base	IgA	IgG roof	—	—	IgG
8	—	—	—	—	IgG roof	—	IgG roof	—

Staining patterns: Roof = roof of blister (epidermal).
Base = floor of blister (dermal).
Comb. = roof and floor of blister (epidermal and dermal).
n.d. = not done.

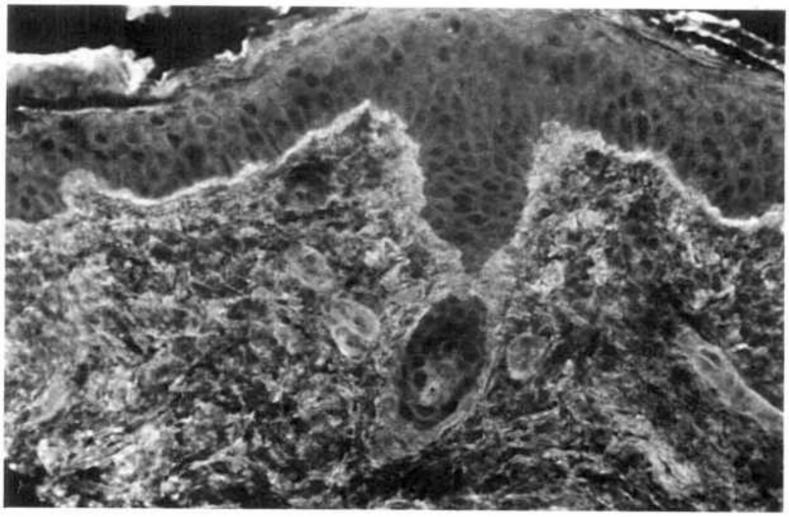

FIGURE 2. Indirect IF with BP serum. Linear deposition of IgG in the BMZ of intact human skin. (Original × 120).

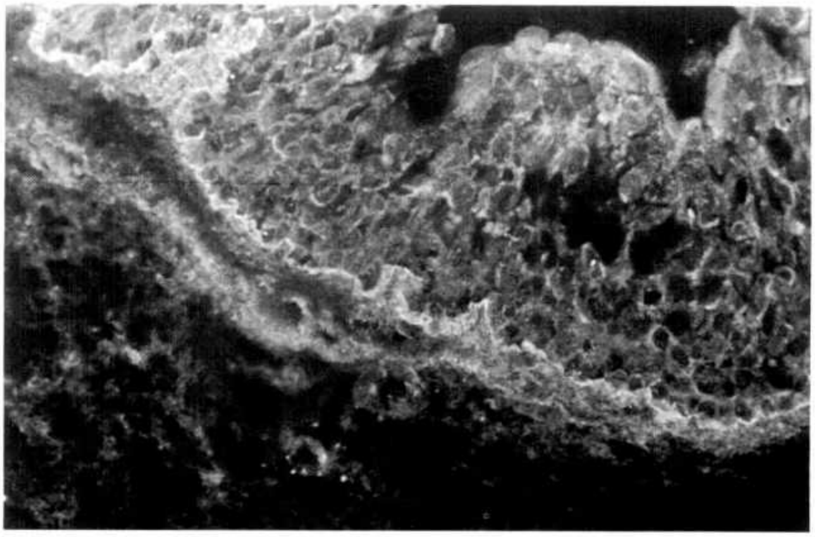

FIGURE 3. Indirect IF with BP serum. Linear IgG staining of the BMZ in conjunctiva. (Original × 120).

Anti-BMZ complement fixing factor was detected in 45% of BP sera using intact substrates and in 25% using separated substrates. Complement fixing factor was detected in 50% of CP sera using oral mucosa; other substrates proved less sensitive.

Pattern of binding

With chemically split substrates we observed three distinct patterns of antibody binding, with

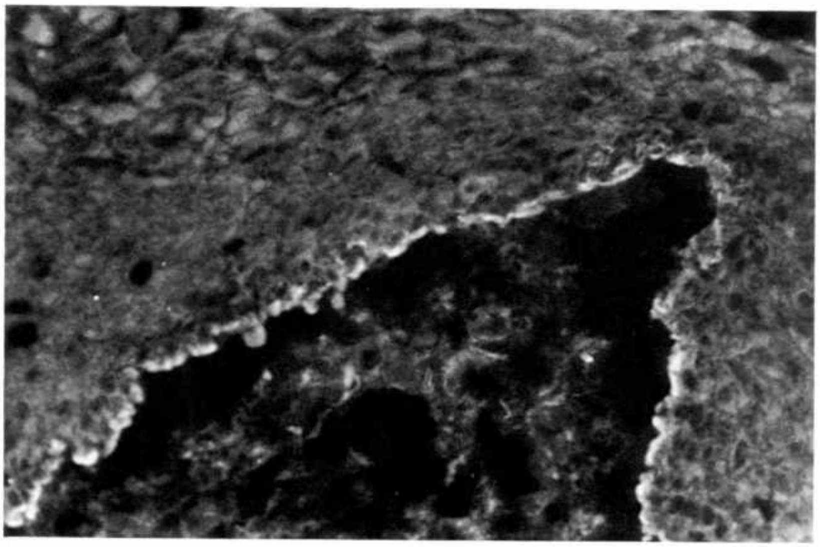

FIGURE 4. Indirect IF with BP serum. Linear IgG staining of roof of split in split skin (epidermal pattern). (Original × 200).

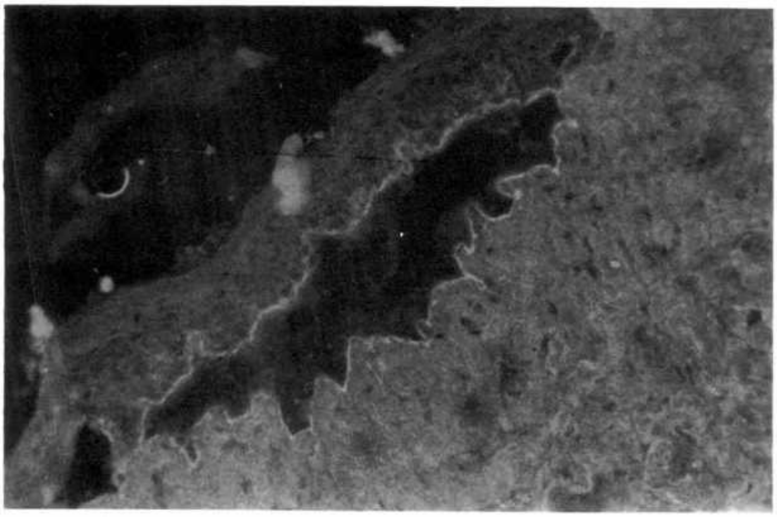

FIGURE 5. Indirect IF with BP serum. Linear IgG staining of split skin showing combined (dermal and epidermal) pattern. (Original × 80).

staining either of the roof of the split (epidermal) (Fig. 4), the floor of the split (dermal) or both (combined) (Fig. 5) (Tables 2 and 3).

Immunoglobulin class

The incidence of the different immunoglobulin classes is given in Table 4. The class of immunoglobulin bound to split substrate was different to that bound to intact substrate in 35% of BP sera and 25% of CP sera.

TABLE 4. Detection of different antibody classes on different substrates

		Number of sera positive					
	Antibody class	Human skin	Split skin	Oral mucosa	Split oral mucosa	Vaginal mucosa	Split vaginal mucosa
BP sera	IgG	10	18	8	12	10	11
	IgM	3	2	0	1	0	3
	IgA	1	0	2	0	1	0
	C_3	5	3	8	2	5	5
CP sera	IgG	2	3	2	4	2	2
	IgM	2	0	1	0	0	1
	IgA	0	0	1	0	0	0
	C_3	2	2	4	0	1	1

Controls

All 10 control sera were negative on all substrates.

DISCUSSION

Bullous pemphigoid and cicatricial pemphigoid have similar histological and immunological appearances, but they differ clinically, with CP usually affecting the mucous membranes to a greater extent, although there is evidence of extensive mucosal disease in some cases of BP.[8]

Anti-BMZ antibodies can be detected in the sera of both groups of patients using indirect IF. In BP these antibodies are directed against BPA, a normal constituent of the BMZ of stratified squamous epithelia. The antigen in CP has not been identified with certainty, although it may be identical to BPA.

Innoculation of animals with BP serum has been found to induce lesions morphologically and histologically similar to human BP.[12,13]

Some previous studies have suggested that the substrate used in the indirect IF technique can influence the results. Bystryn[14] observed variation in antibody reactivity with different substrates; no single substrate was found to be best for the detection of anti-BMZ antibodies, and optimal results were obtained using monkey and guinea pig oesophagus in parallel.

Monkey oral or oesophageal mucosa has previously been the substrate of choice for routine indirect IF[11,15] although use of primate tissue provides only an approximation to human antigen. On biological grounds it would seem more appropriate to test human autoantibodies against human tissue. However, when human skin is used as a substrate for IF, results may be influenced by the site from which the skin is obtained, possibly due to regional and individual variation in the expression of BPA.[17] We chose skin from the flexor surface of the forearm, a site thought to have a high concentration of BPA.

Previous studies have demonstrated wide variation in the detection of circulating antibodies in sera from patients with BP and CP; 60–80% in BP[18,19] and 10–20% in CP.[20] In addition to using monkey oesophagus, we have studied the use of human skin and mucous membranes, both intact and split using 1 M NaCl, as substrate in an indirect IF assay. It has previously been shown that 1 M NaCl separates skin though the lamina lucida leaving BPA on the epidermal side of the split and the lamina densa on the dermal side.[7] A previous study using human tissue substrates, including mucous membranes, failed to show an increase in the sensitivity of indirect IF testing in CP.[21]

In the present study, use of split tissue as substrate improved the sensitivity of the IF technique, increasing antibody detection to 95% in BP and 50% in CP. This may be due to improved exposure of the antigen in the split tissue. We believe that use of tissue which has been artificially 'blistered' through the lamina lucida is more appropriate pathologically. Scaletta's original work on the use of 1 M NaCl to separate skin[9] involved incubating skin at 4°C. We found that separation was easier and the necessary incubation time shorter if the tissue was maintained at room temperature. This may reflect continued protease activity at room temperature which would be inactivated at the lower temperature.

Conjunctiva appeared to be a promising substrate but proved technically difficult to work with. The samples available were so small that we found it impossible to split them through the lamina lucida. Conjunctiva is not easy to obtain in large amounts and thus is not practical as a routine substrate.

In both diseases we identified three patterns of staining using separated tissue, epidermal, dermal and combined. Gammon *et al.*[6] reported similar results on separated skin and suggested that BP sera producing combined or epidermal patterns may have anti-BMZ antibody specificities which differ from those of BP sera which produce only dermal staining. Immunoelectron microscopy showed that antibodies which produced epidermal and combined patterns were anti-lamina lucida antibodies while antibodies which produced only dermal staining patterns were anti-sublamina densa antibodies.[6] This may be important for full categorization and accurate diagnosis. In the analogous situation of Goodpasture's glomerulonephritis, the site of deposition of the anti-glomerular BMZ antibodies determines the clinical features and ultimate prognosis.[22] Gammon *et al.*[6] have suggested that patients with the dermal pattern may have epidermolysis bullosa aquisita (EBA). However, Logan *et al.*[23] have shown that this is not invariably so.

There were no clinical differences between our three groups of patients and we felt that none of the dermal group had EBA. Additionally, we demonstrated a difference in the antibody class detected on split substrate. This suggests that the chemical separation of the epidermis from the dermis modifies the antigenic molecules present in the BMZ. BPA is an intracellular macromolecule produced by basal keratinocytes and liberated into the lamina lucida. This may be a vulnerable position which makes the molecule susceptible to conformational changes when dermal-epidermal separation occurs. This may expose cryptic antigenic epitopes which are seen experimentally after incubation with 1 M NaCl and pathologically in the subepidermal blistering condition. Our separated tissue, therefore, may be providing a substrate which is more comparable to the situation in lesional skin.

The range of antibodies which we have demonstrated is not seen routinely. However, most autoimmune diseases consist of an oligoclonal response and the observation that more than one antibody is involved is not unusual. This does, however, raise problems in defining BPA in terms of reactivity with BP sera, and suggests that application of monoclonal antibody techniques and immunoblotting may be more accurate.

We suggest routine use of split human skin and mucosa as tissue substrates for indirect IF as a practical and reliable method for the diagnosis of BP and CP.

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Lichen sclerosus et atrophicus and autoimmunity—a study of 350 women

R.H.MEYRICK THOMAS, C.M.RIDLEY*, D.H.MCGIBBON** AND M.M.BLACK**

Departments of Dermatology, St Bartholomew's Hospital, West Smithfield, London,* Whittington Hospital, Archway, London and **St Thomas' Hospital, Lambeth Palace Road, London UK.

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SUMMARY

A study of autoimmune related phenomena in 350 women with histologically confirmed lichen sclerosus et atrophicus revealed that 21·5% had one or more autoimmune related diseases, 21% had one or more first degree relatives with an autoimmune-related disease, 42% had an autoantibody at a titre >1:20, and 59·5% had one or more of these autoimmune-related phenomena.

No statistically significant differences in the natural history of lichen sclerosus et atrophicus were demonstrated between those patients with autoimmune-related phenomena and those without.

The aetiology and pathogenesis of lichen sclerosus et atrophicus (LSA) is unknown. It is likely that the causation is multifactorial, and there is evidence to implicate genetic, hormonal and autoimmune-related factors in some patients.[1–7]

Whilst an increased incidence of autoimmune-related disorders has been demonstrated in both women and men with the disease[5–7] and an increased incidence of autoantibodies has been shown in women with LSA[4–5], the relevance of these findings is unclear.

As only a proportion of patients with LSA exhibit a tendency to autoimmune-related problems, the present study was carried out to determine whether there are any identifiable differences in the clinical features of LSA in those women in whom autoimmune-related phenomena can be demonstrated compared with those in whom autoimmune-related phenomena are not found.

METHODS

Patients

Three hundred and fifty women with clinically typical LSA were studied. A standardized history was taken for each patient and a full examination carried out. In all patients the diagnosis

Correspondence: Dr R.H. Meyrick Thomas, Department of Dermatology, St Bartholomew's Hospital, West Smithfield, London EC1A 7BE, UK.

was confirmed histologically. Evidence of autoimmune-related diseases was taken from the patients' personal and family history (first degree relatives) and from clinical examination.

Measurements were made of thyroid and liver function, full blood count, blood and urinary sugar, and anti-thyroid (microsomal and cytoplasmic) antibodies, anti-gastric parietal cell antibodies, anti-nuclear antibodies, anti-smooth muscle antibodies and anti-mitochondrial antibodies.

The patients were grouped according to the presence or absence of the following four autoimmune-related phenomena: a personal history of autoimmune disease, a family history of autoimmune disease, presence of autoantibody (titre > 1:20), or any of these three criteria.

Statistical analysis

Using chi-squared statistics (or Fisher's exact test where appropriate) patients with each of the four autoimmune-related phenomena were compared with those without that phenomenon with respect to four aspects of the natural history of LSA: site or sites involved, age at onset, onset in relation to menarche and the menopause and development of squamous carcinoma within LSA.

RESULTS

Clinical features

At the time of the study, the mean age of the patients was 56 years (range 4–91 years) and the mean duration of symptoms of LSA was 10·5 years (range 1–65 years); the mean age at onset of symptoms of LSA was 45·5 years (range 1–90 years). Those patients who were symptomless and were diagnosed as a chance finding, were excluded from the analysis of age at onset of LSA and duration of symptoms. Squamous cell carcinoma developed in 17 patients (5%). The clinical features are listed in Table 1.

TABLE 1. Sites involved with LSA, and age of onset

	Percentage of patients
Sites affected	
Vulval + Perianal skin	44·0
Vulval skin alone	38·0
Vulval + Perianal + Trunk skin	9·5
Vulval + Trunk skin	6·0
Trunk skin alone	2·5
Age at onset (years)	
Pre-puberty	9·0
Post-puberty–20	4·5
21–30	12·0
31–40	10·5
41–50	15·5
51–60	25·5
61–70	14·5
71–80	8·0
>80	0·5
Pre-menarche	9·0
Post-menarche, but pre-menopause	41·0
Post-menopause	50·0

Autoimmune related phenomena

Personal history of autoimmune related disease. Seventy-five patients (21·5%) had one or more autoimmune-related diseases as listed in Table 2; 10 patients had two autoimmune-related diseases and one patient had three.

The development of thyroid disease, pernicious anaemia or diabetes mellitus occurred before the onset of symptoms of LSA in 16 patients and after the onset of symptoms of LSA in the other 15 patients. In only one patient did more than one of these conditions (thyroid disease, pernicious anaemia and insulin dependent-diabetes mellitus) coexist.

Family history of autoimmune-related disease. Seventy-four patients (21%) had one or more first degree relatives with an autoimmune related disease (Table 3); four patients had two first degree relatives with one or more autoimmune-related diseases.

Autoantibodies. One hundred and forty seven patients (42%) had one or more autoantibodies at a titre of > 1:20 (Table 4); 26 patients had two autoantibodies and one patient had three.

TABLE 2. Autoimmune related diseases in patients with LSA

	Patients	
	No.	%
Alopecia areata	32	9
Vitiligo	21	6
Thyrotoxicosis	10	6
Hypothyroidism	12	6
Pernicious anaemia	6	2
Diabetes mellitus (insulin dependent)	3	1
Bullous pemphigoid	2	1
Systemic lupus erythematosus	1	1
Primary biliary cirrhosis	0	1

TABLE 3. Autoimmune related disease in first degree relatives of patients with LSA

	Patients	
	No.	%
Thyroid disease (hyper- or hypo-thyroidism)	25	7·0
Pernicious anaemia	17	5·0
Diabetes mellitus (insulin dependent)	15	4·5
Alopecia areata	14	4·0
Vitiligo	7	2·0

TABLE 4. Autoantibodies in patients with LSA

Autoantibody	Patients No.	Patients %
Anti-thyroid antibody	88	25·0
Anti-nuclear antibody	36	10·0
Anti-gastric parietal cell antibody	33	9·5
Anti-smooth muscle antibody	14	4·0
Anti-mitochondrial antibody	4	1·0

All autoimmune-related phenomena. Two hundred and nine patients (59·5%) had at least one autoimmune-related phenomenon (i.e. a personal or a family history of an autoimmune-related disease or an autoantibody). Sixty-one patients (17·5%) had two autoimmune-related phenomena, and 13 patients (4%) had all three.

Relationship between autoimmune phenomena and natural history of LSA

No statistically significant differences could be demonstrated between those patients with, and those without, any of the four categories of autoimmune-related phenomena, with respect to the site or sites of LSA involvement, age at onset, onset of LSA in relation to menarche and the menopause or the development of malignancy in LSA affected skin.

DISCUSSION

Previous work has demonstrated the association between LSA and autoimmunity. Wallace[1] observed that 3·8% of patients with LSA had vitiligo and suggested that an association between LSA and autoimmunity might exist. It was then demonstrated that 40% of female patients had anti-thyroid antibodies and 44% anti-gastric parietal cell antibodies.[4] Subsequently it was reported that 34% of female patients with LSA had a personal history of autoimmune disease (12% having thyroid disease, 10% pernicious anaemia, 10% alopecia areata, 6% diabetes and 4% vitiligo), a significantly greater proportion than in a control group of women.[5] A significantly higher incidence of autoimmune related diseases was also found in men with LSA compared with controls.[6,7]

Previous studies have been either of relatively small numbers[4–6] or have included patients without histological confirmation of the diagnosis of LSA.[1] We have found a much lower overall incidence of autoimmune related phenomena in our large group of female patients than has been reported previously in women with LSA. Only 21·5% of our female patients with LSA had a personal history of an autoimmune disease. Of those autoimmune diseases of significance to the patients' health we found less than half the incidence previously reported,[5] only 6% of our patients had thyroid disease, 2% pernicious anaemia and less than 1% diabetes. It has been suggested previously that LSA is associated with primary biliary cirrhosis;[8] while four of our patients had anti-mitochondrial antibodies, none had developed primary biliary cirrhosis.

We also found a lower incidence of both autoantibodies and a family history of autoimmune disease. Only 42% of our patients had any autoantibodies compared with 74% previously reported.[5] Whilst anti-thyroid antibodies were commonest, the occurrence of both these and anti-parietal cell antibodies was much lower than previously reported[4,5] (25% of patients had anti-thyroid antibodies and only 9·5% anti-gastric parietal cell antibodies). Only 21% of patients reported having one or more first degree relatives with autoimmune related disease (this may represent an underestimate of the true incidence as it relied solely on patients' knowledge and memory).

As it is important to diagnose thyroid disease, pernicious anaemia and diabetes as early as possible, it has been argued that patients with LSA should be followed up to detect the occurrence of these conditions sooner rather than later.[5] We previously addressed this problem in a group of 50 women with LSA who were over the age of 60 at the time of study (an older population was deliberately chosen in order to maximize the number of patients in whom hypothyroidism or pernicious anaemia might have developed).[9] We concluded that follow-up of patients specifically for the purpose of detecting the development of autoimmune diseases after the diagnosis of LSA had been established was not worthwhile. In our present study we have found that only 31 (9%) of our patients had thyroid disease, pernicious anaemia or insulin dependent-diabetes mellitus, and that only 15 of these developed the condition after the onset of LSA symptoms. Furthermore, of these, only four were detected on account of the study, having been unrecognized previously; three cases had very early hypothyroidism and one pernicious anaemia.

As the mean duration of symptoms of LSA in our 350 patients was 10·5 years at the time of the study, we estimate that only one patient who had developed thyroid disease, pernicious anaemia or insulin dependent-diabetes mellitus would be found during approximately every 250 patient years of follow-up from the time of onset of symptoms of LSA. Whilst we consider that these findings confirm our previous conclusion that regular follow-up specifically for the purpose of screening for these diseases is unjustifiable, we do believe that patients with LSA (and their doctors) should be advised that there is a small risk of the development of these conditions. Clearly the need for follow-up in order to detect malignant change is a separate issue.

There may be differences in the pathogenesis and natural history of a disease when it occurs in association with autoimmune-related phenomena compared with when it does not. An example of this is diabetes mellitus. Type 2 diabetes mellitus, in which resistence to normal or elevated circulating insulin levels develops, arises in middle aged patients in whom there is no increased incidence of autoimmune-related phenomena.

Type 1 diabetes mellitus, which develops due to inadequate insulin production secondary to autoimmune mediated destruction of pancreatic β-islet cells, has a different clinical presentation and natural history, and requires different therapy.

In view of these differences between Type 1 and 2 diabetes, we wondered whether some light might be shed on the aetiology of LSA by seeking differences in the natural history of the disease in those patients with autoimmune-related phenomena compared with those without. We hoped that any such differences detected might help to clarify the role of autoimmunity in the pathogenesis of LSA. However, our study has demonstrated no significant differences in the behaviour of LSA in those patients with autoimmune related phenomena compared with those without, and accordingly has failed to elucidate the relevance of autoimmunity to the disease process.

Our observations of a rather lower incidence of autoimmune related phenomena in patients with LSA than has previously been considered, together with the similarities in the natural history of LSA irrespective of the presence or absence of accompanying autoimmune related phenomena, lead us to believe that autoimmunity is not a factor of major importance in the pathogenesis of lichen sclerosus et atrophicus. This is supported by previous reports of the HLA tissue types of patients with LSA[10-12] which have failed to demonstrate any association between the disease and HLA-B8 or DRW3, the tissue types found to have an increased incidence in individuals with diseases considered to have an autoimmune basis to their pathogenesis.[13]

The significance of the association between autoimmunity and LSA is still unexplained, and any clue to the pathogenesis of LSA that this association might hold remains elusive.

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Candida onychomycosis—an evaluation of the role of *Candida* species in nail disease

R.J.HAY*, R.BARAN†, M.K.MOORE* AND J.D.WILKINSON‡

*Department of Medical Mycology, Institute of Dermatology, United Medical and Dental Schools of Guys and St Thomas's Hospitals, Lisle Street, London WC2, U.K. †Dermatology Unit, Centre Hospitalier, Cannes, France and ‡Wycombe General Hospital, High Wycombe, Bucks, U.K.

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SUMMARY

Nail infections caused by *Candida* species are normally associated with chronic paronychia or chronic mucocutaneous candidiasis (CMCC). However, the role of *Candida* in the pathogenesis of other primary nail dystrophies has been questioned in view of their response to antifungal therapy alone. In the present study of 86 patients with primary nail dystrophies from which *Candida* was isolated, three patterns of nail involvement were found. Nineteen patients, of whom 17 had CMCC, had total dystrophic onychomycosis of at least two nails. The second group consisted of 27 patients with paronychia and lateral onycholysis. In a further 40 patients, who did not have paronychia, *Candida* was isolated from nails showing primary distal and lateral onycholysis. These changes were mainly seen in patients with peripheral vascular disease, particularly Raynaud's disease, or Cushing's syndrome. Nail biopsies from patients in the latter two groups confirmed the presence of yeasts and mycelium in the nail plate and 17 (46%) of those receiving antifungal therapy with ketoconazole or itraconazole showed complete clearance of the nail dystrophy. Good responses to therapy were seen more frequently in patients with peripheral vascular disease or Cushing's syndrome of whom 15 (72%) recovered; distal erosion of the nail plate, mycelium in the nail plate on biopsy or direct microscopy together with the isolation of *C. albicans* were all associated with good responses to antifungals. In addition to patients with CMCC or paronychia, therefore, *Candida* appears to be a significant pathogen in some patients with primary onycholysis of the finger nails, particularly where there is underlying peripheral vascular disease or Cushing's syndrome.

The term onychomycosis includes fungal infections caused by primary nail pathogens which invade the healthy nail plate, as well as those which follow secondary invasion in patients with pre-existing nail disease, particularly onycholysis or onychogryphosis. Nail plate invasion is distinct from colonization of the subungual space, where the organisms are present but do not penetrate nail keratin. While the concept is useful clinically, the distinction between primary

Correspondence: Dr R.J.Hay.

and secondary nail pathogens is by no means clear cut. Dermatophytes are normally classified with the former, although in some patients fungal nail invasion appears to have followed the development of onycholysis. By contrast, certain fungi such as *Aspergillus terreus* and *Acremonium* species are secondary pathogens; on occasions both of these can be identified in dystrophic nails by direct microscopy and isolated in culture.[2] Invasion of the nail by non-dermatophytes, as opposed to colonization, can normally be inferred by the presence of hyphae in nail material on direct microscopy and by reisolation of the same organism on multiple occasions. Many fungi, however, do not fit in with this classification so conveniently. *Scopulariopsis brevicaulis*, for instance, has features of both primary and secondary nail pathogens and can be found in both previously healthy as well as diseased nails.[3] The predilection of this organism for great toe nails in those without overt primary nail disease suggests that trauma to the nail or onycholysis might be a factor in initiating invasion. In all these examples the main issue from a clinical standpoint is whether removal of the organisms with antifungal therapy will lead to significant improvement of the nail dystrophy.

The role of *Candida albicans* in the pathogenesis of nail disease is an example of this complex problem. Onychomycosis due to *Candida* species is thought to be restricted to patients with chronic mucocutaneous candidiasis (CMCC)[4] or as a secondary event in paronychia. However, the role of *Candida* in the pathogenesis of nail dystrophy ought to be reconsidered in the light of the observation that patients with some forms of nail dystrophy not associated with either CMCC or paronychia from which yeasts can be isolated, may be cured by treatment with an oral antifungal drug alone.[5] Recovery is associated with the disappearance of the fungi from the nail. This suggests that *Candida* may play a significant role in the development of nail dystrophy in some patients.

For this reason we have studied patients with different nail dystrophies from which *Candida* species have been isolated. We have compared the clinical, mycological and histological features and response to antifungal drugs. The object of this study was to assess the significance of *Candida* in nails and to define the indications for using antifungal therapy.

METHODS

Patients

Patients were seen in the outpatients department of St John's Hospital for Diseases of the Skin, London, U.K. or in the Dermatology clinic of the Centre Hospitalier in Cannes, France. A full clinical examination was carried out in each case and evidence of dermatological or internal disease recorded, supported by the appropriate investigations. Nail clippings were taken for direct microscopy and culture. In addition, in some individuals, nail biopsies were taken using a technique described previously,[6] alternatively, nails removed after application of 40% urea were examined histologically. All six nails examined in this way subsequently regrew, but remained infected. Sections from the nail material were stained with haematoxylin and eosin or periodic acid Schiff stains.

The pattern and extent of the nail dystrophy were noted. Dystrophic nails with positive fungal cultures were classified according to the following scheme;[4] total dystrophic onychomycosis (TDO) — complete involvement of the nail plate; distal and lateral subungual onychomycosis (DLSP) — invasion of the distal or lateral nail borders; proximal subungual onychomycosis (PSO) — invasion of the nail plate spreading from the proximal nail fold. No cases of superficial invasion of the nail plate, superficial white onychomycosis were seen. The nail dystrophies were further divided into different groups by the presence or absence of paronychia. A control group of dystrophic nails associated with clinically recognizable skin disease was included.

Mycology

Nail clippings were softened in 30% potassium hydroxide using gentle heat where necessary. The presence of budding yeasts or mycelium or both was noted. Nail material and subungual debris removed with a dental probe were cultured on Sabouraud's dextrose agar with and without cycloheximide at 25°C. Plates were examined after 2 to 3 days, but kept for up to 3 weeks. *Candida* species isolated were identified using standard laboratory methods, including germ tube production, morphology on rice agar/Tween medium, carbohydrate assimilation/fermentation tests and the API 20C *Candida* identification system.

Treatment

Where appropriate oral antifungal treatment was given. The drugs used were oral ketoconazole or itraconazole given in doses of 200 mg and 100 mg daily, respectively. The itraconazole was kindly provided by Janssen Pharmaceuticals, Wantage, U.K. Therapy was continued until clinical and mycological recovery was achieved. The latter was considered to have been reached when both direct microscopy and culture became negative. Liver function tests were carried out on patients receiving ketoconazole before therapy, after 2 and 4 weeks of therapy and monthly thereafter. Patients on itraconazole had regular full blood counts, liver function and electrolyte studies at similar intervals.

RESULTS

A total of 86 patients with finger nail dystrophies, which were culture positive for *Candida* species and for which no other cause was apparent on clinical examination, were included in the study. These were compared with 46 patients with finger nail dystrophies due to pre-existing skin disease, mainly psoriasis (32 patients). In 28 patients the latter diagnosis was based on an association between typical nail changes[8] and lesions of psoriasis elsewhere on the skin, but in four, distal onycholysis and extensive nail pitting had developed without typical psoriatic skin plaques.

Patients were divided into four groups for assessment: those with nail dystrophy associated with clinically recognizable skin disease (Group A, controls), patients with total dystrophic onychomycosis (Group B) or those with primary onychodystrophy, mainly onycholysis, with paronychia (Group C) or without paronychia (Group D). A pre-requisite for including any patient in group, B, C or D, was at least one positive culture of *Candida* sp. from the nails.

Of the 86 patients in groups B, C and D there were 16 males and 70 females, mean age 47 years (range 7–81 years). There were 46 patients in the control group (Group A), 22 males and 24 females mean age 51 years (range 12–75 years)

Group A—patients with onychodystrophy associated with existing skin disease

All patients in this group had recognizable nail dystrophies associated with non-infective skin disease. Details are given in Table 1. Of 46 patients, 32 had psoriatic nail dystrophy consisting of onycholysis (32), pitting (31), with co-existent psoriatic plaques (22) or a past history of psoriasis (6). Many reported tenderness of the affected nail plate. Only a small proportion of this group (26%) had *Candida* in nail cultures and mycelial elements were seen in only three. Six of the yeasts isolated were species of *Candida* other than *C. albicans*.

TABLE 1. *Candida* species, isolated from patients with nail disease associated with dermatoses (Group A)

Dermatosis	No. of patients	Microscopy	Organisms isolated
Psoriasis	32	Yeasts (5)** Yeasts + mycelium (2)	*C. albicans* (4) *C. guilliermondii* (2) *C. parapsilosis* (1) *C. krusei* (1)
Miscellaneous*	14	Yeasts (5) Yeasts + mycelium (1)	*C. albicans* (2) *C. guilliermondii* (2)

None of the five psoriatic patients treated with ketoconazole showed a response

*Yellow nail syndrome (4), lichen planus (2), hand eczema (6), 20 nail dystrophy (2).

**Numbers in parentheses are numbers of patients.

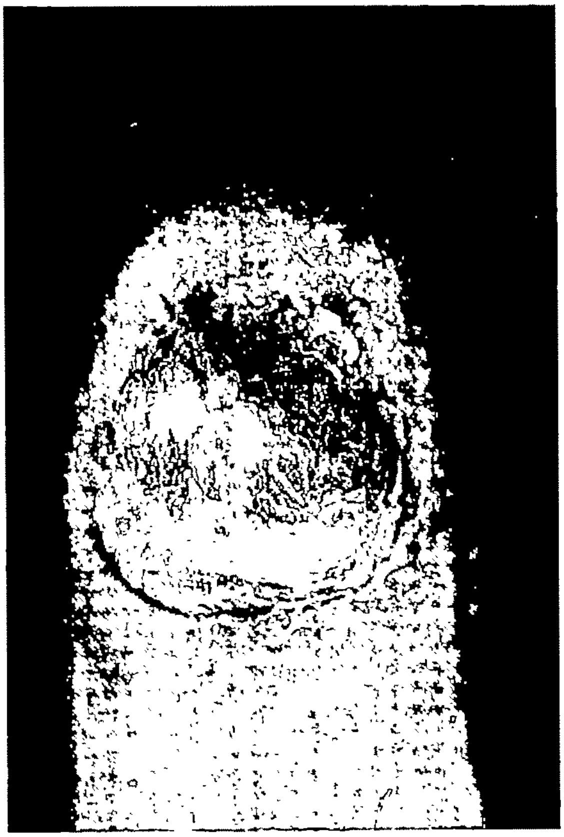

FIGURE 1. Total dystrophic onychomycosis in a patient with chronic mucocutaneous candidiasis. Note the thickened and crumbly nail plate.

Eradication of *Candida* from the nails with ketoconazole was only attempted in five patients, all of whom had psoriasis. Although three of these reported some improvement and lessening of discomfort, the nail dystrophy did not show sustained or significant recovery after a mean period of 5·4 months therapy. In two of these patients the organism isolated from subungual debris, *Candida albicans*, persisted, despite 4 months therapy.

Group B—patients with total dystrophic onychomycosis due to Candida

In 19 individuals at least two nails showed full thickness dystrophy of the nail plate with a varying degree of involvement of the nail fold. Only four of these were males (21%). The mean age of this group was 27·4 years (range 7–64). The main clinical features of the dystrophy were gross hyperkeratosis of the nail plate with distortion of the normal curvature and distal erosion. The plate was opaque, white to yellow or even tan in colour and, in most patients, friable (Fig. 1). Nails not showing total dystrophy had onycholysis with severe erosive changes at the distal margin. In five cases there was considerable hypertrophy of the proximal segment of the plate with the formation of a prominent ridge across this region. In four patients the proximal nail fold was bolstered and a fungal paronychia was confirmed by both microscopy and culture. In one patient this had led to gross swelling of the proximal nailfold with considerable expansion of the terminal phalanx (Fig. 2). Pus was expressed from this area. The numbers of nails affected in patients in this group ranged from three to nine (mean 5).

Details of these cases are listed in Table 2. Seventeen of these patients had chronic mucocutaneous candidiasis (CMCC). In eight the nail dystrophy was secondary to *Candida* endocrinopathy syndrome (hypothyroidism, three; hypoadrenalism and hypoparathyroidism, four; pernicious anaemia, one). Two patients, however, did not have CMCC. One, a 63-year-old woman, had dystrophic changes affecting six finger nails, two of which showed the pattern of TDO. The rest showed onycholysis with distal erosion of the nail plate. She was receiving

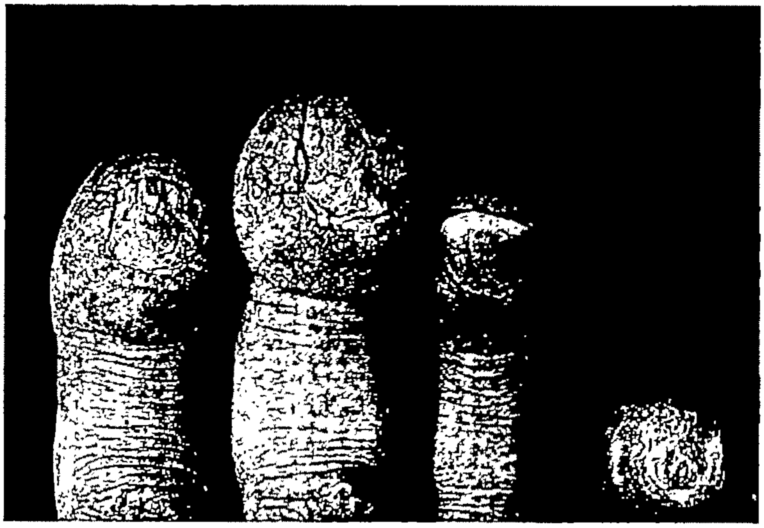

FIGURE 2. Patient with chronic mucocutaneous candidiasis showing involvement of the nail plate and proximal nail fold.

TABLE 2. Total dystrophic onychomycosis due to *Candida* (Group B)

	No. of patients	Microscopy	Culture
CMCC	17	Yeasts + mycelium	*C. albicans* (17)* *C. guilliermondii* (2) *C. parapsilosis* (1)
Cushing's syndrome	1	Yeasts + mycelium	*C. albicans* (1)
SLE	1	Yeasts + mycelium	*C. albicans* (1)

All patients responded to ketoconazole therapy
*Numbers in parentheses are numbers of patients

prednisolone, 10–20 mg daily, for the treatment of allergic bronchopulmonary aspergillosis. She had clinical features of Cushing's syndrome with easy bruising, moon facies and hypertension. A second cushingoid patient had TDO affecting three finger nails with onycholysis of the remaining four abnormal nails. She was aged 53 and was receiving prednisolone in doses up to 40 mg daily and azathioprine 100 mg daily for systemic lupus erythematosus.

Mycological findings are also listed in Table 2. *C. albicans* was isolated from all patients and both mycelium and yeasts were seen on direct microscopy in all. No nail biopsies were taken from this group. All patients achieved complete remission on ketoconazole in a mean period of 7·2 months. Only two of this group relapsed during a mean post-treatment follow-up period of 3·8 years. Both responded to retreatment. During the post-treatment period *Candida* sp, but not *C. albicans* were isolated from subungual debris without clinical evidence of relapse. Fifteen of the treated patients showed residual changes in the appearance of the nail such as shortening and increased curvature of the nail plate (9), nail plate thickening (3) and residual swelling of the proximal nail fold in those presenting with accompanying chronic paronychia.

Group C—patients with onycholysis associated with growth of Candida and paronychia

There were 27 patients in this category, 11 males and 16 females, mean age 42·6 years (range 14–74 years). Eleven patients had underlying peripheral vascular disease (Raynaud's phenomenon or Raynaud's disease, six; severe recurrent perniosis, four; and intermittent claudication, one). In most cases the nail dystrophy was limited to a narrow strip of lateral onycholysis spreading from the proximal nail (PSO), and affected a single finger in 16. This was associated with proximal nail fold swelling. More extensive onycholysis was seen in eight patients and in 11 there was involvement of more than one nail, although only finger nails were affected. In no case was there thickening of the nail plate although terminal erosion was seen in two patients. Some reported pain from the affected nails. The mycological findings and response to therapy are shown in Table 3. Nail biopsies were taken from five patients in this group. In four of these fungal hyphae and yeasts were seen in the ventral surface of the nail plate, but penetration of fungal hyphae extended for only a short distance into the keratin. Partial nail removal with 40% urea was carried out on a further three patients and again yeasts and mycelial elements were seen in the ventral nail plate.

TABLE 3. *Candida* associated with lateral nail dystrophy in patients with paronychia (Group C)

Predisposing disease	No. of patients	Microscopy	Culture	No. of patients achieving remission in response to ketoconazole
Raynaud's phenomenon or disease	6	Yeasts + mycelium (4)* Yeasts (2)	*C. albicans* (5) *C. tropicalis* (1)	4 of 5
Other peripheral vascular disease	5	Yeast + mycelium (4) Yeasts (1)	*C. albicans* (9) *C. guilliermondii* (1)	4 of 5
No underlying disease	16	Yeasts + mycelium (14) Yeasts (2)	*C. albicans* (13) *C. guilliermondii* (2) *C. krusei* (1)	8 of 10

* Numbers in parentheses are numbers of patients.

TABLE 4. *Candida* associated nail dystrophy (distal or lateral) in patients without paronychia or primary nail disease (group D)

Predisposing disease	No. of patients	Microscopy	Culture	No. of patients achieving remission in response to antifungals
Raynaud's phenomenon or disease	12	Yeasts + mycelium (10)* Yeasts (2)	*C. albicans* (11) *C. guilliermondii* (1) *C. parapsilosis* (1)	8 of 10
Other peripheral vascular disease	8	Yeasts + mycelium (5) Yeasts (3)	*C. albicans* (7) *C. guilliermondii* (2)	4 of 8
Cushing's syndrome	3	Yeasts + mycelium (2)	*C. albicans* (2) *C. parapsilosis* (1)	3 of 3
No underlying disease	17	Yeasts + mycelium (4) Yeasts (13)	*C. albicans* (13) *C. parapsilosis* (2) *C. guilliermondii* (2) *C. tropicalis* (2)	2 of 12

* Numbers in parentheses are numbers of patients.

Group D—patients with nail dystrophy without paronychia

There were 40 patients in this group, of whom 37 were female. The mean age was 51 years (range 19–81 years). There was no evidence of coexisting skin disease to account for the changes in any of these patients. All had positive cultures for *Candida* sp. In all patients at least two and up to seven nails were affected. The most common abnormality was onycholysis of the lateral border or distal edge of the nail which was seen in all cases (Fig. 3). In 10 cases there was terminal erosion of at least one nail. There was no paronychia, but many of the patients reported that their nails were friable and split easily. Thickening of the plate was minimal. These changes were

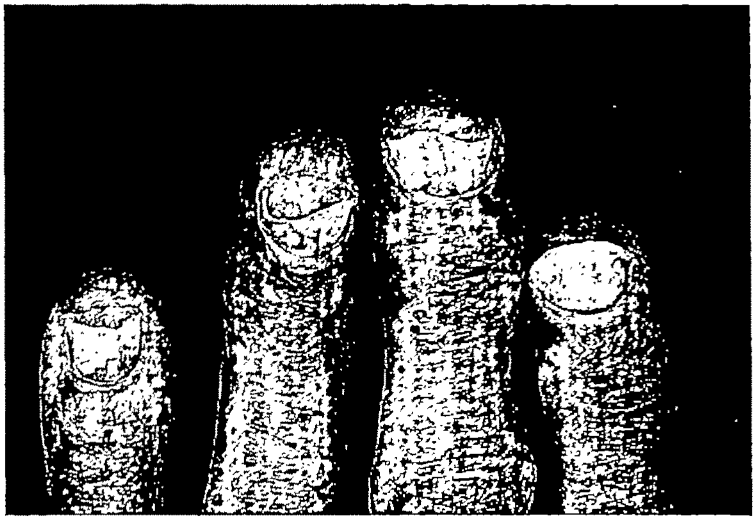

FIGURE 3. Distal and lateral subungual onychomycosis due to *Candida*. The patient had long-standing Raynaud's disease.

indistinguishable from the milder form of dystrophy seen in patients in Group B. Thirty five patients in Group D reported pain, described usually as an ache, from the nail plate on pressure. This was more noticeable in newly dystrophic nails. In over half the cases (58%) there was an underlying disease, usually peripheral vascular disease (20) or Cushing's syndrome (3) (Table 3). One of the latter patients had been treated with systemic corticosteroids for asthma. The other two had Cushing's syndrome associated with pituitary oversecretion of ACTH. Nail biopsies or chemical nail removal were performed in 15 cases. In 13 of these, yeasts were seen in

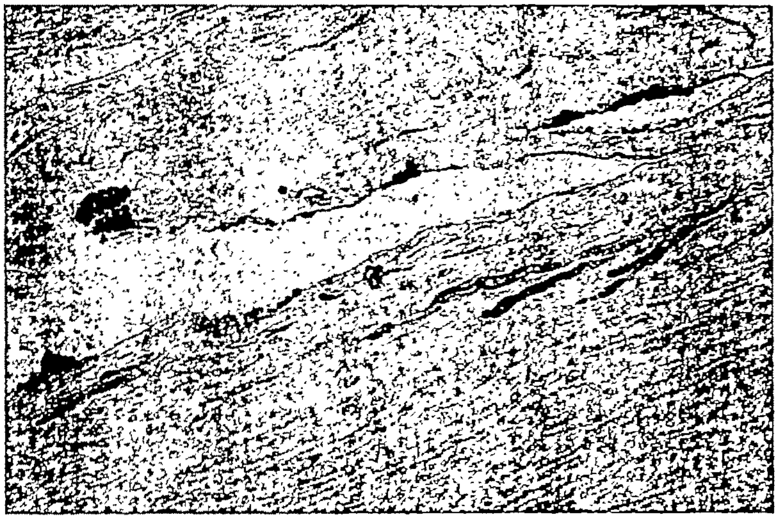

FIGURE 4. Nail biopsy from a patient in Group D. Yeasts and mycelial elements can be seen within the nail plate. (PAS; original × 400).

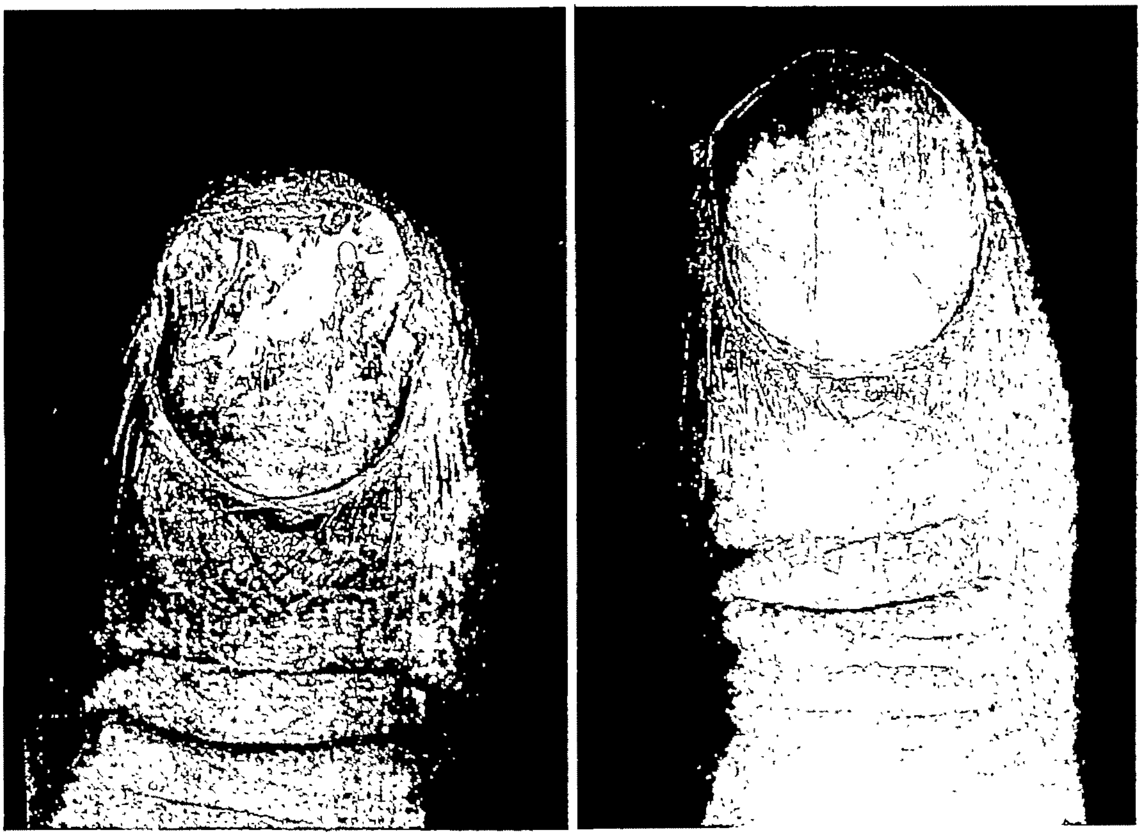

FIGURE 5. (a) Close up of a nail with DLSO due to *Candida*, without paronychia. (b) The same nail after 6 months treatment with itraconazole.

the ventral nail plate and hyponychium and in 10 there were mycelial elements extending into the nail plate (Fig. 4). Of the latter, mycelium had been reported in direct preparations of the nails in 9.

The cultural and clinical features are listed in Table 3. Of 21 patients with underlying disease receiving oral antifungals 15 (72%) achieved clinical and mycological remission with ketoconazole (given to 16 patients) or itraconazole (given to five patients) in mean periods of 6·6 and 7·1 months respectively (Fig. 5a & b). By contrast, only two patients (17%) without underlying abnormalities out of 12 receiving therapy achieved remission with ketoconazole (given to 10 patients) or itraconazole (given to two patients). This difference was statistically significant ($P < 0{\cdot}001$, Student's *t*-test). The presence of *C. albicans* alone in nail material cultured from the patients did not appear to be a predictive factor in determining the likelihood of response to therapy unless mycelial elements were seen on direct microscopy.

DISCUSSION

In this investigation we attempted to assess the relevance of *Candida* isolated from nail material to the development of nail dystrophy by studying a group of 86 patients with culture positive nail disease not attributable to other causes. Nineteen of these patients had total dystrophic onychomycosis affecting one or more finger nails, mainly associated with chronic mucocutaneous candidiasis, all cases being caused by *C. albicans* (Group B). In 27 individuals paronychia was associated with lateral onycholysis extending from the proximal nail fold (Group C). Of these, 41% had underlying peripheral vascular disease. However, in 40 patients distal or lateral onycholysis had developed without paronychia, *Candida* species being isolated from all cases (Group D). The predominant species was *C. albicans* in 83% of cases. All but two patients in this group were female and half had peripheral vascular disease such as Raynaud's phenomenon or perniosis. Of 37 patients in this group who received oral antifungal chemotherapy, either ketoconazole or itraconazole, 17 (46%) achieved clinical and mycological remission. Remission was significantly more likely in those with underlying circulatory disease or Cushing's syndrome. Within a mean follow-up period of 4·4 years (range 0·5–6·4 years) five successfully treated patients relapsed, but responded to a further course of antifungals. In patients with nail dystrophies caused by clinically recognizable skin disease (Group A) only a small proportion harboured *Candida* species of which at least half were not *C. albicans*. None of the five patients in this group who received antifungals achieved remission.

Previously *Candida* has been implicated in nail disease as a primary invader in patients with chronic mucocutaneous candidiasis or secondary to paronychia[4,8] and rarely as superficial white onychomycosis in infants.[8] In one study of 765 patients with *Candida* infection reported from Japan, 25 individuals were found to have candidiasis of the nails with thickening and erosive changes compatible with TDO.[9] Of these 18 had underlying disease including systemic lupus erythematosus (SLE), diabetes mellitus, pemphigus, dermatomyositis, rheumatoid arthritis and histiocytosis X. SLE was a predisposing factor in nine. Biopsies confirmed the presence of fungal hyphae in the nail plate. In the present investigation two patients without CMCC had developed total nail dystrophy. One of these had SLE, the other was receiving systemic corticosteroid therapy. However, in addition to these groups, our results would indicate that *Candida* may be implicated in the process of nail plate separation in some patients whose clinical abnormalities are confined to onycholysis or terminal nail plate erosion. These patients respond completely to oral antifungal therapy. From our study the important factors pointing to such a mechanism are the presence of terminal nail plate erosion, mycelial elements on direct microscopy with the isolation of *C. albicans* and the presence of symptomatic peripheral vascular disease or Cushing's syndrome. Histology performed on 15 nail biopsies from this group confirmed that the mycelium had penetrated into the nail plate from the ventral surface in 10 patients. However, this was also recognized on direct microscopy of nail clippings in all but one patient. This suggests that while nail biopsy is clearly useful in confirming the presence of invasive fungal infection of the nail it does not appear to be essential. Yeasts are often seen to be clustered along the ventral nail plate, but if sections are screened carefully the presence of hyphal elements in the nail plate can be confirmed.

In Group D it is difficult to explain the response to therapy without assuming that the fungus had played some role in the development of onycholysis. It seems likely that the fungus had invaded nails showing some degree of onycholysis, as a secondary pathogen, a phenomenon sometimes seen in dermatophytosis. The main nail changes recorded in patients with peripheral vascular disease such as Raynaud's phenomenon, seen in many of this group, are longitudinal ridging, increased splitting, thinning of the nail plate and onycholysis.[10] It is, therefore, possible that under appropriate conditions *Candida* colonizing the subungual space can penetrate the nail plate. The clusters of yeasts along the ventral surface of the nail plate found in the biopsies, might support this argument.

One reason for doubting the pathogenic potential of *Candida* in nail disease has been the difficulty of relating presence to pathology. Attempts to demonstrate nail plate invasion by inoculation of *C. albicans* under the distal free margin of the nail or proximal nail fold have not been successful.[11] However, it has been shown that *C. albicans* will penetrate nails *in vitro* in the presence of certain nutrients such as glucose.[12] The process is very slow although invasion is quicker in nail samples from children. It has also been shown that *C. albicans* can release amino acids from culture media containing keratin as the sole nitrogen source in the presence of glucose.[13] Once again the process is slow. The nature of the enzyme is unknown but *Candida* can produce an inducible proteinase[14] and has been shown to invade keratinized structures such as hair in disseminated candidiasis seen in heroin addicts.[15] The complete destruction of the nail plate seen in patients with CMCC also suggests that keratin digestion occurs. All the studies cited above used *C. albicans* and there is as yet no evidence that the other species of *Candida* can split keratin.

While Cushing's syndrome has been recorded in some patients with dermatophytosis[16] and pityriasis versicolor,[17] it has not been described previously, to our knowledge, in association with *Candida* nail infections. In the present study, five patients, of whom two had endogenous Cushing's syndrome associated pituitary hyperfunction, showed nail plate invasion with *Candida*. In the other three patients the nail dystrophy followed chronic administration of oral corticosteroids. In these cases, after the cause of the Cushing's syndrome had been rectified the nail infection persisted until antifungal therapy had been given. Other forms of nail dystrophy have not been recorded with Cushing's syndrome and the mechanism of invasion remains speculative.

In conclusion, we believe that it is appropriate to treat patients with total dystrophic onychomycosis or lateral onychomycosis secondary to paronychia due to *Candida albicans* with oral anti-*Candida* therapy, although in the latter group similar clinical responses have been seen in patients receiving topical therapy with econazole lotion alone.[18] In addition, certain patients with onycholysis, not due to underlying dermatoses, from which *C. albicans* is isolated may also respond to this therapy. From our study we would suggest that patients in this latter category should receive therapy if they have symptomatic peripheral vascular disease such as perniosis or Raynaud's phenomenon, positive direct microscopy of nail clippings with mycelial invasion together with the isolation of *C. albicans*. It may also be worth treating those patients who meet the latter two criteria but not the first with a trial of antifugal chemotherapy if topical drugs fail to produce a response.

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Epithelial distribution of a myelomonocytic antigen L1 in relation to cutaneous malignancies and melanocytic naevi

T.-Ø.GABRIELSEN, P.BRANDTZAEG, P.S.HOEL AND I.DALE

Laboratory for Immunohistochemistry and Immunopathology, Institute of Pathology, The National Hospital, Rikshospitalet, Oslo, Norway

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SUMMARY

The distribution of L1 antigen in four common skin tumours was examined immunohistochemically in 40 samples of formalin-fixed routine biopsy material. Presence of L1 in the dermis depended on the extent and composition of the inflammatory infiltrate; L1 was found mainly in reactive histiocytes and neutrophils. Intense epidermal staining was seen adjacent to squamous cell carcinoma, basal cell carcinoma and malignant melanoma, but not adjacent to melanocytic naevi. In specimens of melanocytic naevi, L1 staining around pilosebaceous units was weak or absent. Around the three malignant types of lesion, epidermal L1 positivity faded distally—thereby indicating the extent of the tumour. L1 was, in addition, expressed by the most highly differentiated cells in squamous cell carcinomas, whereas the other neoplasms were negative. Antibody to L1 may, thus, be a useful adjunct for distinction between various types of skin tumours.

L1 is a 36·5 kd protein first isolated from granulocytes[1] and subsequently characterized as a specific myelomonocytic antigen.[2,3] It consists of three non-covalently linked polypeptide chains.[1] L1 is mainly a cytoplasmic component, but it is also expressed on the surface of circulating neutrophils and monocytes.[2] Its distribution in mononuclear cells present in various tissues is largely restricted to infiltrating macrophages (reactive histiocytes.)[3] In addition, it occurs in normal squamous epithelium of mucous membranes (oropharynx, oesophagus and vagina and cervix)[4], but is absent from normal epidermis except in the pilosebaceous units.[4,5] The epidermal L1 antigen has physicochemical properties similar to the myelomonocytic L1 protein.[4]

A recent investigation demonstrated that enhanced epidermal L1 expression is a frequent although variable finding in several inflammatory skin diseases.[5] Since the L1 antigen is well

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Correspondence: Dr T.-Ø. Gabrielsen LIIPAT, Rikshospitalet, 0027 Oslo 1, Norway.

preserved in routine formalin-fixed pathological material,[4] retrospective immunohistochemical studies of stored paraffin blocks can be performed. We report here an investigation of the distribution of L1 in some tumours of the skin.

METHODS

Forty formalin-fixed biopsy specimens from four different tumour categories, as listed in Table 1 were obtained from the routine files of our pathology laboratory. Sections stained with haematoxylin and eosin (H&E) were assessed blind by a dermatopathologist. Only when he confirmed the histopathological diagnosis already given, was a specimen selected for immunohistochemical investigation. All specimens were from adult patients. Control material was obtained as described previously.[5]

TABLE 1. Immunohistochemical staining for L1 in skin tumours

	Epidermal cells			Dermal inflammatory infiltrate			Tumour cells		
Tumour type	+	(+)	–*	+	(+)	–	+	(+)	–
Basal cell carcinoma ($n=10$)	10	–	–	4	–	6	–	–	10
Squamous cell carcinoma ($n=10$)	10	–	–	10	–	–	10	–	–
Malignant melanoma ($n=10$)	10	–	–	7	–	3	–	–	10
Melanocytic naevus ($n=10$)	–	7	3	–	–	10	–	–	10

*+: Distinctly positive; (+): faintly positive; –: negative.

Immunoperoxidase staining

Paraffin sections were cut at 6 μm, dewaxed, and subjected to inactivation of endogenous peroxidase with 0·3% H_2O_2 in methanol for 30 min at room temperature. Then the sections were washed twice in phosphate buffered saline (pH 7·5), rinsed in deionized water and air-dried. Subsequent immunostaining was based on the unlabelled antibody peroxidase antiperoxidase (PAP) method, performed as detailed elsewhere[6], except that a buffer containing 0·2 M sodium acetate, 5 mM calcium chloride and 5 mM magnesium chloride was used for all washes. The substrate reaction was developed with diaminobenzidine (DAB). The sections were finally counterstained with Harris' haematoxylin, mounted in buffered polyvinyl-alcohol (pH 8·0) and observed by light microscopy. Controls for specificity of the staining reactions included omission of the primary antiserum or its application after absorption with purified L1 antigen (final concentration, 0·01 g/l).

Primary antibody

Antiserum to L1 purified from granulocytes was raised in rabbits as detailed previously.[2] After absorption with normal human serum, the selected reagent showed a single strong precipitin line when tested by double diffusion in agar gel against crude leukocyte extract; the only unwanted activity detectable was a faint precipitin line against lactoferrin. The antiserum was therefore absorbed with purified colostral lactoferrin and the IgG fraction was isolated.[2] For immunostaining this fraction was used at 0·009 g/l with 20 h incubation at room temperature.

RESULTS

General observations

As reported previously,[4,5] normal skin was negative for L1 except in some of the pilosebaceous units and areas adjacent to their orifices. Intense diffuse epidermal L1 staining was, however, demonstrated in all pathological specimens except those containing melanocytic naevus cells which showed no or only faint staining. When positive, the epidermis was mostly diffusely stained above the basal layer which was generally negative with the exception of a few scattered cells. In specimens from malignant melanoma where the epidermis was atrophic and the basal cell layer seemed to be lacking, staining was seen throughout all layers. As reported previously,[5] L1 was apparently absent from the granular layer in cases with marked hypergranulosis and dense keratohyalin particles, despite its presence in the cornified layer. When the specimens contained epidermis extending beyond the tumour infiltrates, L1 positivity often stopped abruptly at the border of the neoplasm. However, in some specimens the L1 staining extended beyond the area where the tumour mass or the accompanying inflammatory infiltrate seemed to be located.

Dermal L1 staining depended on the presence of leukocyte infiltration and varied to some extent with the type of lesion present. Some of the sections containing a squamous cell carcinoma had many L1-positive elements, but they were never dominant among the inflammatory cells. Most specimens contained only scattered positive cells unless the lesion was ulcerated; in these cases L1-positive neutrophils were the predominant cell type. Trypsin digestion was not performed in the present study, and some macrophages might, therefore, have escaped from L1 labelling as described elsewhere.[3] Conversely, the staining of granulocytes and squamous epithelium is not greatly affected by trypsin digestion.[3]

Brown colouration due to melanin was an important pitfall in naevi and melanomas but could be identified in the H&E-stained serial control sections. Moreover, a slight difference in colour was observed between melanin and the DAB reaction product. Control sections with omission of primary reagent or with L1-adsorbed primary reagent showed no immunostaining.

Basal cell carcinoma

Epidermal cells just above or adjacent to the tumour infiltrates showed L1 positivity in all 10 specimens examined (Table 1). Epidermis extending beyond the tumour was present in six specimens; it was weakly stained in two and completely negative in four.

The tumour cells were always negative for L1 (Fig. 1). Islets with L1 positivity were ascribed to hair follicles embedded in the tumour infiltrates and could be easily distinguished from the neoplastic tissue. Tumour differentiation into cutaneous appendages was a frequent observation but did not result in L1 positivity. Where the tumour masses were merging with the covering epithelium, a sharp demarcation was seen between the generally intensely stained epidermal cells and the negative tumour cells.

About half of the specimens showed L1 staining of dermal inflammatory cells. A dense inflammatory cell infiltrate was seen in ulcerated lesions but was also prominent in two non-ulcerated specimens.

Squamous cell carcinoma

L1 positivity was present in all 10 specimens examined (Table 1), throughout the epidermis except in the basal layer which was negative or only faintly stained. Only two sections contained epidermal margins beyond the underlying tumour and weak staining was observed in both.

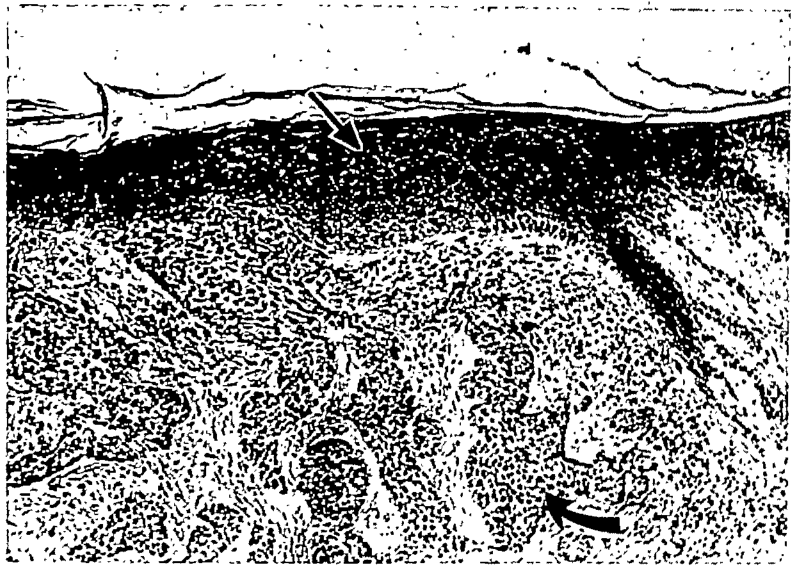

FIGURE 1. Basal cell carcinoma. The epidermis is strongly positive for L1 (straight arrow) in contrast to the negative tumour infiltrate (curved arrow). PAP labelling, counterstained with haematoxylin. (Original × 120).

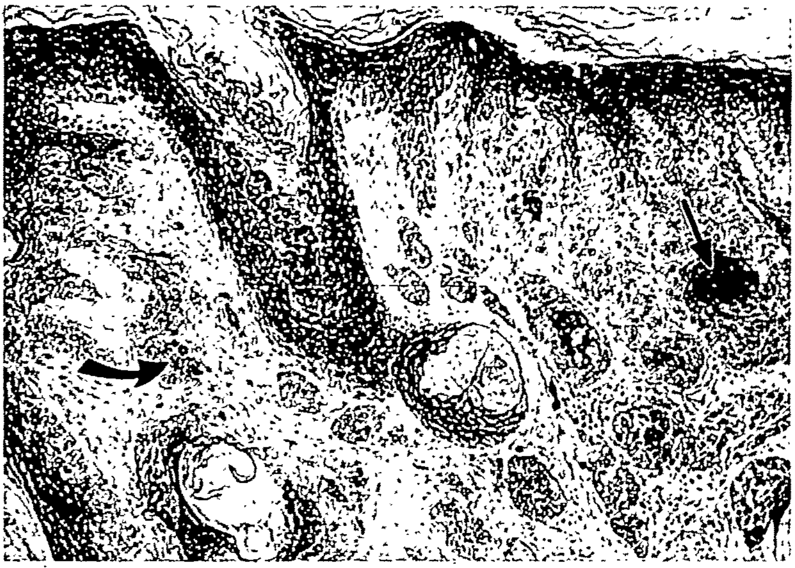

FIGURE 2. Squamous cell carcinoma. The epidermis is strongly positive for L1. The tumour infiltrate is negative (curved arrow), except well-differentiated areas (straight arrow). PAP labelling, counterstained with haematoxylin. (Original × 120).

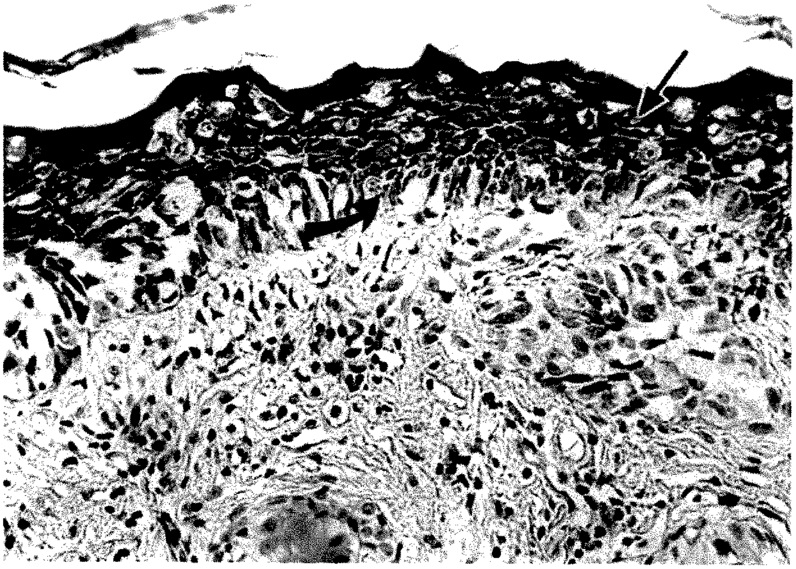

FIGURE 3. Malignant melanoma (superficial spreading type). The epidermis is strongly positive for L1 (straight arrow) whereas melanoma cells are negative (curved arrow). PAP labelling, counterstained with haematoxylin. (Original × 300).

All sections contained L1-positive tumour cells. When considerable keratinization had occurred, positive staining was a frequent finding. Lamellar structures with horn pearls were strongly positive. Less differentiated tumour cells were mostly negative and, therefore, patchy L1 staining was seen throughout the tumour masses (Fig. 2).

The specimens generally contained a dense inflammatory cell infiltrate and many of these cells stained for L1. When ulceration was present, a strongly positive dense infiltrate of pus cells could be seen.

Malignant melanoma

Intense epidermal L1 staining was a consistent finding in these specimens (Table 1; Fig. 3) which comprised two specimens of lentigo maligna melanoma, five of the superficial spreading type, and three nodular melanomas. However, the epidermis was often atrophic adjacent to the tumour. In several specimens the epithelium consisted of only a few cell layers which were visualized as a narrow L1-positive zone, and the basal cell layer seemed to be absent. The L1 staining ceased abruptly at the border of the underlying tumours in six of eight sections with apparently lesion-free epidermal margins (Fig. 4) whereas in two, the perilesional epidermis was weakly stained.

The tumours were completely L1-negative. Tumour cells present in the epidermis were, likewise, negative whether they occurred singly or in nests. Brown staining due to melanin pigment was a frequent finding, but was easily identified.

A substantial inflammatory cell infiltrate was present in four specimens and L1-positivity appeared mainly in histiocytes.

Melanocytic naevus

The epidermis was L1-negative in three of the 10 specimens examined (Table I, Fig. 5). The

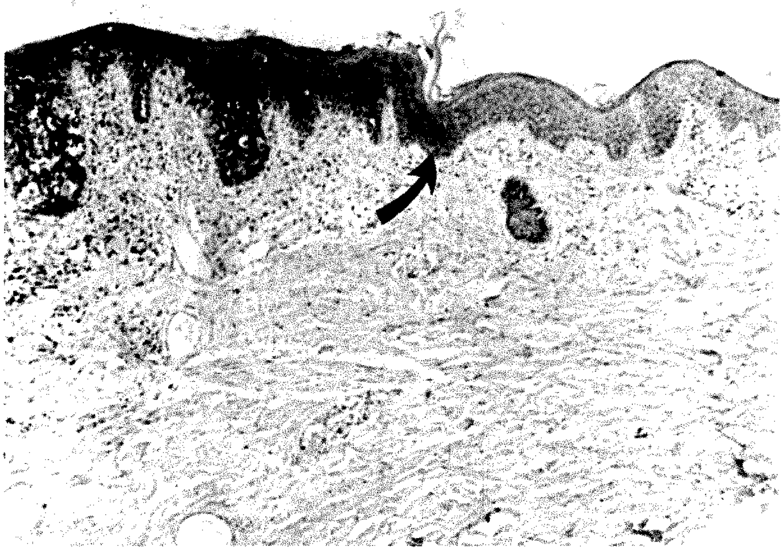

FIGURE 4. Malignant melanoma. Transitional zone (arrow) with L1 positive epidermis overlying the tumour to the left and perilesional L1-negative epidermis to the right (with some scattered positive cells). PAP labelling, counterstained with haematoxylin. (Original × 100).

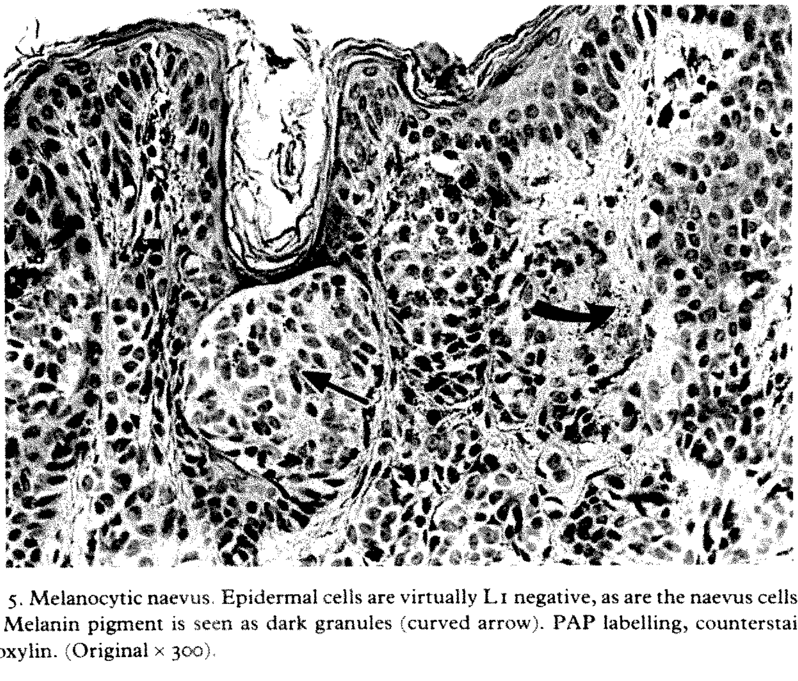

FIGURE 5. Melanocytic naevus. Epidermal cells are virtually L1 negative, as are the naevus cells (straight arrow). Melanin pigment is seen as dark granules (curved arrow). PAP labelling, counterstained with haematoxylin. (Original × 300).

other seven specimens showed only weak or patchy epidermal staining. When pilosebaceous units were numerous in the underlying dermis, epidermal staining was somewhat intensified. This was observed especially in two specimens from the face. Perilesional epidermis was seen in nine cases and it was L1-negative in seven. Two of the specimens seemed to show faint L1-positivity beyond the lesion, but this could be ascribed to uncertainty in determining the exact extent of the tumour.

Melanin was frequently observed, but did not obscure the fact that the tumour cells were completely L1-negative. L1-positive inflammatory cells were not observed in any of the sections.

DISCUSSION

This study showed that epidermal expression of the L1 antigen was intense in malignant melanomas, squamous cell carcinomas and basal cell carcinomas, but only weak or absent adjacent to melanocytic naevi. Epidermal L1 positivity was clearly independent of any underlying inflammatory cell infiltrate, and the suprabasal diffuse staining pattern generally observed did not differentiate between the three malignant tumour types studied.

In specimens from basal cell carcinomas, malignant melanomas and naevi, the epidermal L1 staining was mainly confined to the area of the underlying tumour and often ceased abruptly at its borders. However, the staining could extend into the surrounding epidermis and was then seen to fade gradually until it disappeared completely when the specimens were sufficiently wide. Many biopsies, regretably, did not contain sufficient perilesional skin for such evaluation.

L1 was expressed by highly differentiated tumour cells in squamous cell carcinomas and was frequently demonstrable in areas of keratinization. Tumour tissue from basal cell carcinomas, melanomas and melanocytic naevi, however, was consistently negative. L1 may, thus, be an interesting epithelial tumour marker. Squamous cell carcinomas can, in addition, usually be identified by staining with one or more monoclonal antibodies to keratin.[7] Moreover, antibody to involucrin (a precursor of the protein envelope of the stratum corneum) has been reported to stain squamous cell carcinomas, whereas basal cell carcinomas were virtually negative.[8]

When L1 was present in the dermis it was localized to some of the inflammatory cells. It has been reported previously that L1 is mainly expressed by reactive histiocytes and neutrophils[3], whereas lymphocytes are negative.[2,3] The extent of the inflammatory infiltrate varied considerably and most of the cells were L1-negative. However, as trypsin digestion was not performed we might have missed some L1-expressing macrophages.[3] In some ulcerated lesions of basal cell carcinomas the infiltrate consisted of a large accumulation of neutrophils and the L1 staining was prominent even in the immediate surroundings, indicating leakage from the cells as has been noted previously.[3] In general, most L1-positive leukocytes were seen adjacent to squamous cell carcinomas, whereas the naevi induced no inflammatory reaction.

L1 is, thus, an interesting marker for both leukocytes and squamous epithelium. We are not aware of any other similar cellular antigen, although a rabbit antiserum (S22) raised against human monocytes, apparently showed some resemblance to our anti-L1 antibody.[9] However, in contrast to our antibody, S22 was reported to stain Kupffer cells in normal livers and interdigitating cells in lymph nodes; epithelial staining was not mentioned.[9] Shared reactivity for granulocytes, monocytes and epithelium (not including normal epidermis) has been reported for certain monoclonal antibodies.[10-12] Our antiserum did not react with normal epidermis except in close proximity to hair follicles. However, we have shown that expression of epidermal L1 is enhanced in various skin diseases.[5] Moreover, L1 from psoriatic scales seems to be identical to the myelomonocytic L1 protein.[4]

A recently described monoclonal antibody (Ψ3) that recognizes a 135 kd structural component of maturing keratinocytes, reportedly produced epidermal staining remarkably similar to that of our L1 antibody in various skin lesions, and failed to react with keratinocytes in normal epidermis; reactivity with pilosebaceous units was not mentioned.[13,14] However, the fact that the Ψ3 antibody stained neither inflammatory cells nor oral mucosal epithelium, distinguishes it from our anti-L1 antibody.

The biological role of epithelial L1 expression is still a matter for speculation. One possibility is that synthesis of L1 is turned on when the keratinocytes are in a certain state of proliferation or differentiation. Epidermal L1-positivity may depend both on pathological processes in the dermis (e.g. carcinoma or granuloma) and on disease activity primarily in the keratinocytes. However, its expression is not disease-specific.[5]

There was no strict relationship between the presence of an L1-positive inflammatory dermal infiltrate and epidermal L1 expression. Thus, in nine specimens containing a malignant lesion, the keratinocytes were strongly L1-positive despite absence of L1 staining in the dermis. We have also studied irregular naevi with a rather dense dermal infiltrate containing L1-positive cells, but epidermal staining was not substantially different from that observed adjacent to the melanocytic naevi in the present investigation (Gabrielsen *et al.*, unpublished observations). In addition, the possibility of substantial diffusion of L1 into the epidermis and uptake by keratinocytes seemed unlikely for various other reasons. Brandtzaeg *et al.*,[4] reported that outward diffusion of plasma proteins such as IgG, IgA and albumin tended to follow the intercellular spaces and uptake by epithelial cells was patchy, which contrasts with the diffuse intracellular distribution observed for L1. Moreover, when subepithelial tissue contained numerous macrophages with both L1 and lysozyme, only the latter component appeared in the epithelium.[4] Finally, if epithelial occurrence of L1 depended on diffusion, it should also be generally present in adjacent tumours such as carcinomas and melanomas, in contrast to our observations.

The observation of greatest clinical interest in the present study was the strong epidermal L1 positivity seen adjacent to malignant melanoma cells, contrasting with the weak or absent staining near benign naevi. Whether this disparity is of significant diagnostic importance, is being evaluated in an extensive study which is currently underway.

ACKNOWLEDGEMENTS

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Oestrogen and progesterone receptors in lentigo maligna

M.E.SAWAYA, L.D.GARLAND, M.J.ROTHE, L.S.HONIG, AND S.L.HSIA

Department of Dermatology and Cutaneous Surgery, University of Miami School of Medicine, Miami, Florida, U.S.A.

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SUMMARY

We have measured oestrogen and progesterone binding in specimens of histopathologically confirmed lentigo maligna (LM) lesions excised from five elderly white men, using a dextran-coated charcoal method. Oestrogen binding was observed in four of the five specimens, and progesterone binding in all five. Marginal normal skin showed only non-specific binding for oestrogen and progesterone. The presence of steroid hormone receptors in LM suggests the possibility that hormone responsiveness may be relevant in the transformation of a lentigo maligna into malignant melanoma.

Lentigo maligna (LM) was first described histologically by Hutchinson in 1894,[1,2] and further by Dubreuilh.[3,4] These authors described a slowly enlarging macular lesion with variegated black-brown colouration and an irregular outline, seen commonly on the face and upper extremities, regions of the body typically exposed to the sun. LM is generally seen in the elderly, and long-term follow-up is often difficult since mortality may ensue from other causes unrelated to skin cancers. Clinical reports have suggested that the development of LM tumours follows an unpredictable course. In view of the increasing longevity of the elderly, the mechanism underlying malignant transformation of LM to melanoma warrants investigation. Reports of the presence of oestrogen receptors and progesterone receptors in malignant melanoma[5–8] prompted us to study these receptors in LM tumours.

METHODS

Punch biopsy specimens from five elderly adult white male patients, mean age 69 years (range 64–76 years), were confirmed histopathologically to be LM. The lesions were then excised in toto using a wide excision procedure. The tissues were placed immediately in liquid nitrogen for transport to the laboratory. ^{3}H-labelled synthetic analogues of oestradiol (moxestrol, R2858) and progesterone (promegestone, R5020) (specific activities 87 Ci/mmol) and the non-

Correspondence: S.L.Hsia, University of Miami School of Medicine, Department of Dermatology and Cutaneous Surgery, P.O. Box 016960, R117, Miami, FL 33101, U.S.A.

radioactive synthetic steroids were purchased from Dupont New England Nuclear (Boston, MA, U.S.A.). Skin samples were weighed, and homogenized in 5 ml of 50 mM Tris-HCl, pH 7·4, containing 1 mM EDTA, 1 mM dithiothreitol, and 10 mM sodium molybdate. Homogenates were centrifuged at 164000 *g* for 1 h at 2°C in a Beckman L5-65B ultracentrifuge using a LSW 50·1 rotor. The supernatant cytosol fraction was adjusted to contain 10 mg protein/ml, as determined by the Lowry method[9] and was divided into 200 μl aliquots. The aliquots were incubated for 18 h at 4°C, with varying concentrations (0·25–8·0 nM) of the ^{3}H-ligand with and without 1·6 μM non-radioactive hormone (≥200 fold excess) for determination of non-specific binding. Following incubation, the mixtures were treated with 0·5 ml of a dextran-coated charcoal suspension (0·25% Norit A–0·25% dextran, Sigma Grade C, in 10 mM Tris–HCl, pH 8·0, and 1 mM EDTA) to absorb the unbound steroid. Bound and free steroids were separated by centrifuging the mixtures at 1600 *g* to sediment the charcoal. The radioactivity in the supernatant was determined in a Packard Tricarb liquid scintillation counter with a ^{3}H counting efficiency of 35%. Results were analysed by Scatchard plot and linear least squares regression using a Hewlett-Packard HP 41C with a Statpac programme.

RESULTS

Table 1 show results obtained from analysis of the Scatchard plots for dissociation constants (K_d) and binding capacities in the LM tumours. Four of the five LM specimens exhibited specific oestrogen binding, with a mean binding capacity ± SD of 40·5 ± 7·2 fmol/mg cytosol protein (range 32–49 fmol/mg protein), and mean K_d 1·18 ± 3·9 mM (range 0·73–1·55 nM). All five specimens showed progesterone binding activity with a mean binding capacity of 47·8 ± 19·5 fmol/mg protein (range 27–54 fmol/mg protein), and K_d 1·82 ± 0·66 nM (range 1·26–2·60 nM). In all five specimens, the neighbouring normal tissue showed only non-specific binding of the ^{3}H-ligand.

TABLE 1. Oestrogen and progesterone receptors in cytosol of lentigo maligna lesions

			Oestrogen receptors		Progesterone receptors	
Case no.	Age	Location	K_d (nM)	Binding sites (fmol/mg protein)	K_d (nM)	Binding sites (fmol/mg protein)
1	65	cheek	0·73	32	1·71	43
2	76	cheek*	0·98	38	1·26	27
3	68	mid-back	–	0	1·13	37
4	64	shoulder**	1·55	49	2·60	78
5	74	chest	1·45	43	2·40	54

*LM with melanoma in situ.
**Atypical LM

DISCUSSION

Levels of steroid receptors in melanoma range widely,[5,6,8] but are generally lower than those in tumours of the prostate, breast, or other sex hormone target tissues. Some investigators have failed to detect any high-affinity oestrogen receptors in malignant melanoma,[10] although in general high levels of oestrogen receptors, 63–238 fmol/mg cytosol protein,[5–7] and of progesterone receptors, 11–320 fmol/mg protein[8] have been reported. These values are greater than those we found in LM. However, the dissociation constants for oestrogen and progesterone receptors 0·3–4 nM and 0·5–18 nM respectively were similar to those we found in LM, suggesting that the same receptors may be present, but in greater numbers, in melanoma. The possible presence of multiple binding sites as found in oestrogen target tissues[11,12] requires further investigation.

Invasive disease has been estimated to develop in 30 to 50% of patients with LM.[13–15] According to Michalik *et al.*,[16] transformation of the slow radially growing LM into vertically growing invasive malignant melanoma may occur during a relatively short time interval. It must be noted that frequent follow-up in such cases is not always carried out. Recommended treatment for LM is wide surgical excision of the lesion.[16–18] Our finding of an increase in oestrogen and progesterone receptors in LM compared to normal neighbouring tissue suggests the possibility that steroid responsiveness may be relevant in the tranformation of lentigo maligna into malignant melanoma.

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Polymorphic light eruption sine eruptione

J.S.DOVER* AND J.L.M.HAWK

Department of Photobiology, Institute of Dermatology, St Thomas's Hospital, Lambeth Palace Road, London SE1 7EH, U.K.

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SUMMARY

We describe seven patients, four female and three male, who developed intense pruritus on sun-exposed skin without visible change. The clinical features resembled those of polymorphic light eruption (PLE) without rash. Four patients also occasionally developed typical PLE upon sun exposure, but sun-induced pruritus alone occurred most frequently. No patient was taking any drug therapy. One patient developed similar pruritus following solar simulated irradiation, and one following PUVA therapy. All other laboratory investigations were negative. Treatment with low dose UVB phototherapy or PUVA therapy was effective. The condition, which we have called polymorphic light eruption sine eruptione (PLESE), appears to be a variant of PLE not previously reported.

With the exception of erythropoietic protoporphyria (EPP) and occasional drug photosensitivity, patients with pruritus following sun exposure develop a coexistent skin eruption, the nature of which assists diagnosis. We report here seven patients who developed intense pruritus on exposed skin after sun exposure without visible change or evidence of EPP, or relevant history of drug ingestion.

METHODS

All patients attended the photobiology out-patient clinic of St. John's Hospital for Diseases of the Skin, London, complaining of pruritus following sun exposure. Each underwent routine laboratory studies and assessment of blood, urine and stool porphyrin concentrations, serum antinuclear, anti-Ro and anti-La antibody titres, serum IgE concentration, skin responses to prick tests with 10 common allergens (Bencard) and cutaneous reactions to monochromatic irradiation (Applied Photophysics) and xenon arc solar simulated irradiation (Kratos 2500W).

RESULTS

There were four males and three females, four of skin type I, two of type II and one of type IV.[1] Onset in all cases was in early childhood or young adulthood. The condition had persisted for

Correspondence: Dr J.L.M.Hawk.

TABLE 1. Details of patients studied

Patient no.	Skin type	Age at time of study (years)	Age at onset (years)	Age at presentation (years)	Duration of condition (years)
1	II	22	Infancy	18	22
2	III	28	Infancy	22	28*
3	IV	29	23	23	6
4	II	30	4	29	26
5	III	27	5	19	22
6	II	34	Infancy	30	34
7	II	15	6	11	8**

*Remission between 13 and 18 years of age

**Remission for 1 year prior to the study

between 6 and 34 years in all except two patients, one of whom had been symptom-free for one year after having suffered for 8 years, and the other who had relapsed after 5 years' remission (Table 1).

Pruritus started 15 to 48 h after light exposure and persisted for 1 to 4 days, depending on the length and intensity of sun exposure. Only exposed skin or that under light clothing was affected, with a sharp cut-off at covered skin. Not all exposed areas were always affected, the hands and face being spared in Patients 1, 2, 3 and 6, and the hands in Patient 5. Pruritus did not occur without sun exposure, and tolerance did not develop with repeated exposure (Table 2).

Four patients (Patients 1, 2, 5 and 6) experienced typical polymorphic light eruption (PLE) very rarely after intense sun exposure (usually abroad), but suffered pruritus alone much more frequently. There was a family history of sunlight induced pruritus in Patient 7 and of PLE in Patient 2. No other patient had a family history of light-sensitive disorders or atopy. All patients were otherwise well, except Patient 4 who had suffered from eczema for 1 year. No patient was taking any drug therapy.

TABLE 2. Details of episodes of pruritus

Patient no.	Time between exposure and onset of pruritus (h)	Duration of pruritus (h)	Areas spared
1	4–5*	48–72	Hands, face
2	15 min–48 h*	24–48	Hands, face
3	1–8	24	Hands, face
4	1	48–96	None
5	5–24	48–72	Face
6	1–3	24–48	None
7	1–8	24–48	None

*Sometimes 1–2 days exposure needed initially.

Antinuclear, anti-Ro and anti-La antibodies were not detected in any of the patients. Porphyrin concentrations in blood, urine and stool were normal. Serum IgE levels were normal in the six patients tested and prick tests to 10 common allergens were negative in five of these, but positive in Patient 4.

Monochromatic irradiation tests on back skin with UVB, UVA and visible wavebands were normal. Solar simulated irradiation of back and anterior forearm skin in four patients induced intense pruritus without visible skin changes in Patient 4 only, with the same time course as and mimicking the sunlight-induced pruritus. However, repeat testing 3 months later was negative. One other patient suffered widespread intense pruritus also with the same time course as and mimicking the sunlight-induced reaction during low-dose prophylactic PUVA therapy.

Treatment by sun avoidance was fully effective, high protection factor topical sunscreens helped moderately during sun exposure, although they were not tried during phototherapy, and H1 antihistamines did not work. Low-dose UVB phototherapy or psoralen photochemotherapy (PUVA) was most useful. Patient 5 received a course of 16 treatments with low-dose UVB irradiation over an 8-week period during two successive spring seasons, and Patients 1, 2, 3 and 4, a 6-week course of low-dose PUVA treatment three times weekly in early spring, all with marked improvement during the following summers.

DISCUSSION

All seven patients developed localized intense pruritus on sun-exposed skin, but not elsewhere, with a distinct cut-off at the edge of clothing, strongly suggesting that sun exposure was causative. Differential diagnosis included EPP, drug-induced photosensitivity, lupus erythematosus, light sensitive atopic eczema, solar urticaria and PLE. In all instances except EPP and some drug induced photosensitivity reactions, a typical skin eruption also develops. However, blood porphyrin concentrations were normal in all our patients and none were taking or had taken photosensitizing drug therapy. Lupus erythematosus and its variants were excluded by absence of typical eruption or positive serology, solar urticaria by absence of erythema and wealing and by delayed onset and long persistence of irritation, and classical PLE by absence of rash. Patients with atopic eczema can be light-sensitive[2] but this was essentially excluded in five of six patients by the absence of an eczematous rash or a personal or family history of atopy, by normal serum IgE levels and by negative prick tests. Although Patient 6 did suffer mild atopic eczema throughout the year he always developed the sunlight-associated pruritus without visible skin change.

These patients apparently represent a previously undescribed entity, very probably a subclinical manifestation of PLE. The age of onset, the sex ratio and the nature of the symptoms, occurring hours after exposure, lasting several days and increasing with exposure, all resemble the features of PLE.[2] The production of the symptoms by a solar simulator in one patient and by PUVA therapy in another also supports the aetiological role of sunlight. Furthermore, the lack of reaction to the solar simulator seen in the other patients and the normal irradiation monochromator tests in all the patients are frequent features of classical PLE.[3,4] The patients responded to UV avoidance, sunscreens, low-dose UVB phototherapy or PUVA, therapies which have been found effective in PLE.[4–9] Finally, the fact that four of seven patients occasionally developed typical PLE also strongly supports the suggestion that this condition is a variant of PLE. We are currently investigating how many of our patients with true PLE develop symptoms without signs. We have called the new condition polymorphic light eruption sine eruptione (PLESE), and we consider that this should now be included in the differential diagnosis of intermittent pruritus and of sunlight-induced skin disease. Studies to compare histopathological and pharmacological mediator findings in this condition with those in ordinary PLE are in progress.

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Effect of dietary supplementation with n-3 fatty acids on clinical manifestations of psoriasis

A. BJØRNEBOE, ANNE KLEMEYER SMITH,*
GUNN-ELIN AA. BJØRNEBOE, P.O. THUNE* AND C.A.DREVON

* Department of Dermatology, Ullevål University Hospital, Oslo, Norway

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SUMMARY

In a double-blind, block randomized study we investigated the effect of dietary supplementation with eicosapentaenoic acid in patients with psoriasis. The experimental group received 10 g of fish oil daily containing approximately 1·8 g eicosapentaenoic acid, while the controls were given an isoenergetic amount of olive oil. We found no significant change in the clinical manifestations of psoriasis in either group after 8 weeks of treatment. In the experimental group, the amount of n-3 fatty acids in serum phospholipids was significantly increased at the end of trial as compared to pre-treatment values, whereas the level of n-6 fatty acids was decreased.

Essential polyunsaturated fatty acids are important structural components of cell membranes and precursors for eicosanoids (prostaglandins and leukotrienes). There are two families of essential fatty acids, n-6 (e.g. arachidonic acid) and n-3 (e.g. eicosapentaenoic acid) (Fig. 1).

The cellular content of free arachidonic acid in psoriatic lesions is increased 20 fold as compared to uninvolved epidermis.[1] Free arachidonic acid may be converted by specific enzymatic reactions to prostaglandins, leukotrienes and 12-hydroxyeicosatetraenoic acid (12-HETE) (Fig. 2). These products are all mediators of inflammation. Increased levels of leukotriene B_4 and 12-HETE have been observed in psoriatic epidermis.[2] Leukotriene B_4 formed from arachidonic acid (catalysed by 5-lipoxygenase) is a very potent chemotaxin and promotes the migration of polymorphonuclear neutrophils, whereas 12-HETE is a less potent chemotactic agent.[3] Infiltration of neutrophils, with formation of microabscesses, is one of the histological characteristics of psoriatic lesions. Benoxaprofen, a potent inhibitor of 5-lipoxygenase has been shown to improve psoriasis in several clinical studies,[4] but this line of investigation was discontinued owing to side-effects.

Correspondence: Anders Bjørneboe, National Institute of Forensic Toxicology, P.B. 16 Gaustad 0320 Oslo 3, Norway.

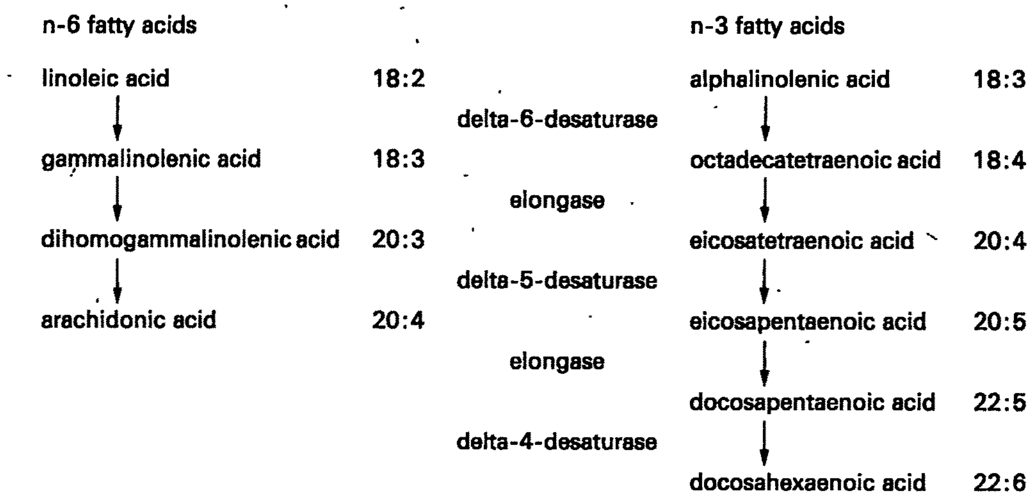

FIGURE 1. Pathways of desaturation and elongation of n-6 and n-3 fatty acids. The n-6 and n-3 fatty acids are desaturated and elongated by the same enzyme systems.

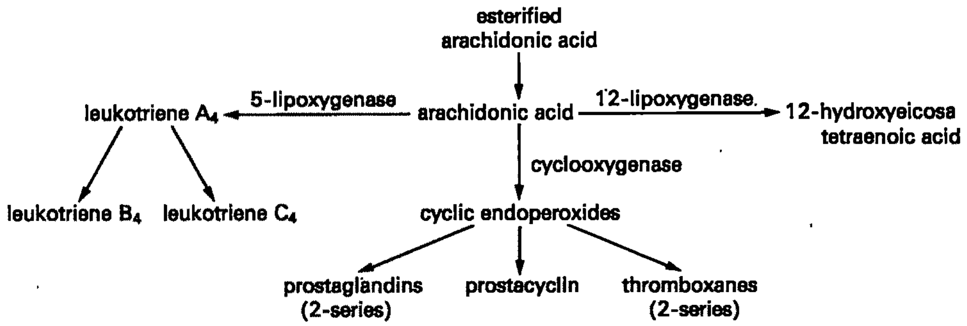

FIGURE 2. Major routes of metabolism of arachidonic acid.

Eicosapentaenoic acid is the precursor of the 3-series of prostaglandins and leukotriene B$_5$ (LTB$_5$). These eicosanoids are less inflammatory than those derived by conversion of arachidonic acid,[5] and LTB$_5$ is a less potent stimulator of keratinocyte proliferation than LTB$_4$.[6] Eicosanoid metabolism is largely dependent on the amounts and types of fatty acid precursors in the diet, and in Western diets n-6 fatty acids are most abundant. Arachidonic acid and eicosapentaenoic acid are cleaved, elongated and desaturated by the same enzyme systems. When n-3 fatty acids are supplied in the diet, competitive inhibition of n-6 eicosanoid metabolism with formation of n-3 eicosanoids may occur.[7,8] This may favour reduced immune reactivity.

We have undertaken a double-blind study to examine the effect of n-3 fatty acid supplementation on psoriatic lesions.

METHODS

Patients

Thirty patients (aged 17–72 years) with stable psoriasis vulgaris were included in the study. To record the severity of psoriasis a clinical scoring system was used.[9] Erythema, infiltration and desquamation were scored separately on the head, trunk, arms and legs on a scale from 0–3 and the scores totalled. Area involved was recorded for the head, trunk, arms and legs, each of these four regions being taken as representing 25% of the total body surface area.

All patients underwent a detailed physical examination and interview by a clinician. Patients were excluded if they were on retinoid treatment, were taking warfarin or salicylic acid or had disease of the gastrointestinal tract or the liver. The ability to comply with dietary supplementation was ascertained, and informed consent was obtained from all participants.

Study design

The patients entered an 8-week, double-blind block randomized trial, starting in January 1986. For 2 months prior to the study, all the patients had stopped using topical steroids. The patients kept up their regular diet during the study, but they were told not to consume cod liver oil. Patients in the experimental group received daily 10 capsules of MaxEpa® (Seven-Seas, Hull, U.K.) containing 10 g of fish oil, and patients in the control group received 10 placebo capsules (olive oil) daily.

The fatty acid composition of MaxEpa® is approximately 18% eicosapentaenoic acid (EPA), 12% docosahexaenoic acid, 30% total n-3 fatty acids and 3% n-6 fatty acids. Each capsule contained 1 IU of alpha-tocopherol (vitamin E) as antioxidant, 100 IU of vitamin A and 10 IU of vitamin D.

Each patient's psoriasis was assessed before the trial and after 4 and 8 weeks by the same clinician using the scoring system described above.

The final clinical score for each symptom in each patient was subtracted from the initial symptom score and the experimental and control groups compared.

Compliance was measured by counting the capsules consumed and measuring the serum phospholipid fatty acid composition at baseline and after 8 weeks. An increased serum concentration of EPA was expected in all patients in the experimental group.

Laboratory measurements

Blood samples were taken at baseline and after 8 weeks for measurement of full blood count, erythrocyte sedimentation rate (ESR), fatty acid profile in the serum phospholipids, cholesterol, triacylglycerol, kreatinine, albumin, alanine-aminotransferase (ALAT), gamma-glutamyltranspeptidase (GGT), alpha-tocopherol and selenium. Bleeding time was also measured in all patients at baseline and after 8 weeks of treatment.

Alpha-tocopherol (vitamin E) in serum was measured by high performance liquid chromatography combined with fluorometry.[10] This vitamin is an essential antioxidant, required to protect the structure of polyunsaturated fatty acids from oxidative damage in cell membranes.[11]

For analysis of the fatty acid pattern in serum phospholipids, 0·4 ml serum was extracted with 8 ml chloroform/methanol (2:1, v/v). The lipid extracts were separated by thin layer chromatography on silica gel plates using petroleum ether/diethyl ether/acetic acid (113:20:1, v/v/v) as developing solvent. The distinct band of phospholipids was scraped off the plate into glass-stoppered tubes and converted to methyl esters of fatty acids by a mixture of benzene, methanolic HCl and acetondimethylacetal (Fluka AG, Buchs SG, Switzerland). The methyl esters were separated and measured by gas liquid chromatography using an apolar capillary column (bonded phase, vitreous silica, 0·22 ID, length 30 m, S.G.E., Victoria, Australia). The gas chromatograph (Carlo Erba Strumentazione, Fractovap Series 2150, Italy) was equipped with a flame-ionization detector and the oven temperature was programmed to rise rapidly from 60°C to 220°C (39°C/min), then slowly (0·7°C/min) to 250°C. Retention times and peak areas were automatically computed by a Hewlett Packard 3390 integrator. Identification of individual methyl esters was performed by comparison with authentic methyl ester standards (Supelco Inc., Bellefonte, PA. U.S.A.) analysed under the same conditions. Other laboratory measurements were performed routinely at Ullevål University Hospital, Oslo, Norway.

Statistics

The data were analysed using the Mann-Whitney test and Spearman's rank correlation coefficient.

RESULTS

Thirteen patients in the experimental group (eight men and five women, mean age 40 years, range 17–72 years) and 14 in the control group (nine men and five women, mean age 39 years, range 22–71 years) completed the 8 week trial. There were two dropouts in the experimental group and one in the control group because of inability to swallow the capsules.

We found no significant difference between the experimental and control groups at baseline with respect to clinical scores for psoriasis (Table 1). The total score for erythema, infiltration and desquamation was 14·4 (range 8–29) and 14·3 (range 6–23) in the experimental and control groups respectively. We found no significant change in the clinical scores during the trial in either group.

At baseline the mean serum concentrations of alpha-tocopherol (± SD) were 23·8 ± 5·3 µmol/l and 21·1 ± 3·5 µmol/l in the experimental and control groups, respectively (Table 2). After 8 weeks of MaxEpa® the mean serum concentration of alpha-tocopherol in the experimental group was significantly reduced as compared to pre-treatment values ($P < 0{\cdot}01$), whereas no significant change was found in the control group.

In the experimental group, total n-3 fatty acids in serum phospholipids (20:5, 20:3, 22:6), was significantly increased at the end of the trial as compared to pre-treatment values (Table 3). MaxEpa® also caused a significant fall in the arachidonic acid (20:4, n-6) and linoleic acid (18:2, n-6) content of serum phospholipids. The arachidonic acid content was reduced by 15% at the

TABLE 1. Clinical assessment of psoriasis before and after the trial

	Experimental group				Placebo group			
	Before		After		Before		After	
	mean	range	mean	range	mean	range	mean	range
Erythema	6·7	4–11	6·6	4–12	6·2	2–11	6·1	2–11
Infiltration	1·2	0–8	0·8	0–5	0·3	0–3	0·2	0–2
Desquamation	6·5	2–12	6·8	1–12	7·8	3–11	6·5	1–11
Area	32	8–80	33	8–80	36	10–80	33	10–80

TABLE 2. Bleeding time and serum alpha-tocopherol before and after the trial. Values are means ± SD

	Experimental group		Control group	
	Before	After	Before	After
Bleeding time (min)	4·5 ± 2·1	4·5 ± 2·1	4·2 ± 2·0	4·3 ± 1·3
Alpha-tocopherol (μmol/l)	23·8 ± 5·3	21·8 ± 5·0*	21·1 ± 3·5	21·5 ± 4·0

* $P < 0{\cdot}01$ compared with pretreatment value (Mann-Whitney test).

TABLE 3. Fatty acid pattern in serum phospholipids in experimental and control groups before and after the trial. Values are percentages of fatty acids identified in the serum phospholipids (means ± SD)

Fatty acid	Experimental group		Control group	
	Before	After	Before	After
12:0	0·4 ± 0·2	0·6 ± 0·2	0·4 ± 0·1	0·6 ± 0·3
14:0	0·3 ± 0·1	0·5 ± 0·1	0·3 ± 0·2	0·4 ± 0·1
16:0	30·8 ± 1·8	30·5 ± 2·2	30·2 ± 1·9	29·3 ± 1·7
16:1	1·0 ± 0·1	0·9 ± 0·2	0·9 ± 0·2	1·0 ± 0·2
18:0	14·8 ± 1·5	16·2 ± 1·6	14·6 ± 1·4	15·6 ± 0·7
18:1, n-9	10·3 ± 1·6	9·6 ± 1·1	10·2 ± 1·5	11·1 ± 1·1
18:1, n-11	2·8 ± 1·3	3·7 ± 0·8	2·5 ± 1·2	3·0 ± 0·9
18:2, n-6	22·8 ± 2·2	18·4 ± 2·4*	23·5 ± 2·2	23·8 ± 2·6
20:3, n-6	2·5 ± 0·5	1·6 ± 0·3*	2·6 ± 0·4	2·9 ± 0·9
20:3, n-3	0·5 ± 0·2	1·0 ± 0·4*	0·4 ± 0·2	0·6 ± 0·1
20:4, n-6	7·8 ± 1·4	6·6 ± 1·6†	8·3 ± 1·5	7·0 ± 1·0
20:5, n-3	1·7 ± 0·7	5·0 ± 1·8*	1·6 ± 0·7	1·2 ± 0·4
22:6, n-3	4·3 ± 1·2	5·1 ± 1·1†	4·2 ± 1·1	3·4 ± 0·7

* $p < 0{\cdot}005$, † $p < 0{\cdot}01$ compared with pre-treatment values (Mann-Whitney test).

end of the trial. In contrast, we found no significant change in phospholipid fatty acid pattern in the control group.

Routine laboratory measurements performed at baseline were normal and none of the parameters changed significantly during the trial (data not shown). The bleeding time did not increase in either group during the trial (Table 2).

DISCUSSION

In open trials Ziboh *et al.*[12] and Allen *et al.*[13] reported a moderate clinical improvement in psoriasis after dietary supplementation with fish oil. In the present double-blind controlled study, we were not able to confirm their findings. We used an amount of eicosapentaenoic acid which can be obtained from daily intake of fat fish, whereas these authors used a five to sixfold greater amount of n-3 fatty acids.

Eicosapentaenoic acid in serum phospholipids increased in all the patients receiving MaxEpa®. This objective measure confirmed satisfactory compliance in the experimental group. The n-3 fatty acids must have been incorporated into serum phospholipids at the expense of n-6 polyunsaturated fatty acids, although n-6 fatty acids continued to be supplied in the diet. This finding is in accordance with previous reports,[14] and may be explained by competition between n-3 and n-6 fatty acids for the 2-position (carbon atom number 2 in the glycerol backbone) in phospholipids. Thus, an altered pattern of cyclooxygenase-derived compounds due to changes in precursor fatty acid stores may develop.

Bleeding time did not change significantly in the experimental group during the trial. This is in accordance with some previous reports, even though a prolonged bleeding time may be expected after n-3 fatty acid supplementation.

In the experimental group receiving n-3 fatty acids, we found a significantly reduced serum concentration of alpha-tocopherol at the end of trial. Highly polyunsaturated fatty acids require effective antioxidants to protect their structure. Each capsule of MaxEpa® contained 1 mg alpha-tocopherol as antioxidant. Accordingly, it is possible that the antioxidant protection exerted by alpha-tocopherol supplied with the capsules was suboptimal.

The n-3 fatty acids may exhibit anti-inflammatory properties by modulating eicosanoid metabolism. We recently reported beneficial effects of n-3 fatty acids in patients with atopic dermatitis,[15] and previously supplementation with fish oil was reported to be associated with improvement in some of the clinical manifestations of rheumatoid arthritis.[16] Inflammation is crucial in the pathogenesis of these illnesses as well as in psoriasis. Using moderate doses of n-3 fatty acids, we found psoriatic lesions to be unresponsive to dietary supplementation with fish oil. However, in view of the findings of Ziboh *et al.*[12] and Allen *et al.*,[13] we consider that the possible role of n-3 fatty acids in the treatment of psoriasis should be tested more extensively. Both higher doses and longer periods of treatment should to be examined in double-blind studies.

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Evaluation of a therapeutic concentration of dithranol in the mouse-tail test

MECHTILD HOFBAUER, PAULINE M. DOWD, JANET ATKINSON,
M. WHITEFIELD* AND M.W. GREAVES

Institute of Dermatology, United Medical and Dental Schools, Lisle Street, London and * Dermal Laboratories Limited, Gosmore, Hitchin, Herts., U.K.

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SUMMARY

A study of dithranol ointment in the mouse-tail test showed that 0·2% dithranol ointment induced a granular layer and orthokeratosis in mouse-tail epidermis. This provides further evidence of the value of the mouse-tail test in evaluating anti-psoriatic activity of topical preparations.

Parakeratosis and the absence of a granular layer are characteristic features of epidermis in the involved skin of psoriasis. Although no animal model of psoriasis exists[1], the normal adult mouse tail has been used to evaluate anti-psoriatic activity[1–5]. The adult mouse tail has proved useful in this respect as, unlike normal human skin, it possesses a granular layer and exhibits orthokeratosis only in perifollicular areas of the epidermis. The areas of epidermis between the follicles in adult mice, referred to as scale areas of epidermis, do not possess a granular layer and exhibit parakeratosis. The orifices of hair follicles occur in regions of the epidermis termed hinged junctions,[1] as shown in Figure 1. Induction of a granular layer and orthokeratosis in areas of scale between the hinges, previously characterised by parakeratosis and absence of a granular layer, are taken to be indicators of anti-psoriatic activity. Using the mouse-tail test, a variety of topical preparations including coal tar, a synthetic tar and dithranol have been assessed for anti-psoriatic activity.[2–5] The study on dithranol[5] was performed using a 10% concentration in an ointment containing 5% wool fat in yellow soft paraffin. Despite its undoubted efficacy in psoriasis, dithranol in the above formulation failed to induce a granular layer, although it did cause a thickening of the epidermis. To explain this surprising result, the investigators suggested that, at the high concentration used in their experiments, the mitotic stimulatory effect of dithranol might have over-ridden effects relating to differentiation. Since dithranol is most commonly used in concentrations as low as 0·1–1·0%[6], we have investigated the effects of a standard ointment containing 0·2% dithranol in the mouse-tail test.

Correspondence: Dr M. Whitefield, Dermal Laboratories Limited, Tatmore Place, Gosmore, Hitchin, Herts., SG4 7QR U.K.

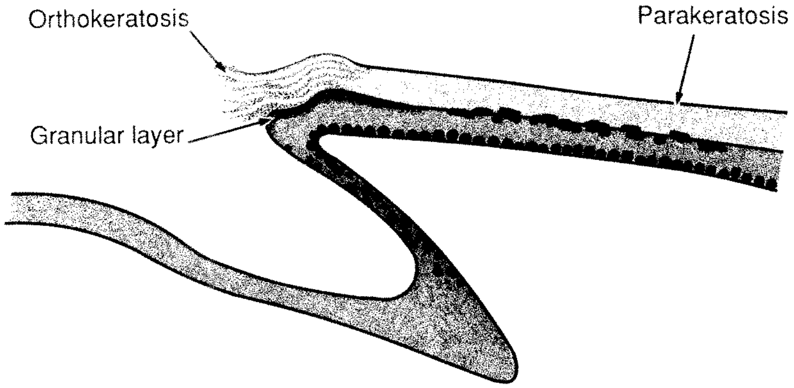

FIGURE 1. Diagramatic representation of mouse tail epidermis to show orthokeratotic hinge area with underlying granular layer and parakeratotic inter-hinge scale.

METHODS

Dithranol (0·2%) incorporated into white soft paraffin BP was compared with the vehicle alone and with equal rubbing of the tails of the mice in the absence of dithranol ointment or vehicle. Groups of ten albino mice, (five male, five female) of the St John's hairless HrHr genotype, aged 7 to 9 weeks and weighing approximately 25 g, were allocated randomly to each of the three treatment groups A, B and C. Ten mice in each group was considered the necessary number to detect an effect in the light of previously reported experiments[2,3] and advice from Dr R. Wrench. Dithranol ointment 0·2% was applied daily for 7 consecutive days to the tails of the mice in Group A. Vehicle alone was applied in an identical manner to the tails of the mice in Group B. The tails of the mice in Group C were rubbed twice with the fingers against the grain of the scale each day at the same time as the applications of ointments were made to the other two groups of mice. All mice were examined blind for erythema and staining of their tails at each treatment. The mice were sacrificed 24 h after the last treatment and the tail skin was cut along the ventral surface, stripped off and flattened for 10 min in 70% alcohol. Histological processing was carried out as previously described[7]. Wax embedded sections of the mouse tail skin were cut, mounted and stained with haematoxylin and eosin. Two non-adjacent sections from each mouse tail were examined for the presence of a granular layer, orthokeratosis and parakeratosis and scored blind by two observers: −, granular layer and orthokeratosis found only in their normal distribution, + a sparse patchy granular layer and orthokeratosis present when the plane of sectioning resulted in a short inter-hinge distance, + +, a continuous granular layer and orthokeratosis present along the whole of the length of inter-hinge scale areas. Epidermal thickening was assessed qualitatively as present or absent.

RESULTS

Clinical effects

No deterioration in the general condition of the mice in any group was observed. Erythema of the tail of one mouse in Group A was noted on days 3 and 4, and by day 5 all mice in this group exhibited erythema. No tail erythema was observed in any mouse in Groups B and C. Application of the ointment resulted in softening in the consistency of the scale of the tails of all the mice in Groups A and B. No difference in the consistency of the scale was observed in Group C.

Histology

The results are summarized in Table 1. In Group A, a continuous granular layer and orthokeratosis in non-hinge areas appeared in seven mice (Fig. 2a) and an intermittent granular layer occurred in the remaining mice when the inter-hinge distance was small. In Group B, an intermittent granular layer was observed in very few scales in one mouse and a continuous granular layer occurred in another mouse when the plane of sectioning resulted in a short inter-hinge distance in several scales. Neither a granular layer nor orthokeratosis was observed in the inter-hinge areas of scale of any of the mice in Group C (Fig 2b). Qualitative estimations of epidermal thickening showed that this was apparent in Group A but not in Groups B or C.

DISCUSSION

Therapeutic concentrations of dithranol induce a granular layer and restore orthokeratosis in involved skin of psoriasis. Whilst the mouse tail is not an animal model of psoriasis, it has been used to screen drugs for anti-psoriatic activity by their ability to induce a granular layer and orthokeratosis in previously parakeratotic areas of epidermis, termed scales, normally occurring between the hair follicles in adult mice. In the present study dithranol (0·2%) in white soft paraffin produced a positive result in this test, whereas white soft paraffin alone and the action of rubbing the tails failed to produce any consistent changes. Thickening of the epidermis was also

TABLE 1. Effect of a 0·2% dithranol ointment, vehicle and rubbing alone on the induction of a granular layer and orthokeratosis in mouse-tail epidermis

Mouse No.	Group A Dithranol	Group B Vehicle	Group C Rubbing only
1	++	+	−
2	++	−	−
3	++	−	−
4	++	−	−
5	++	++	−
6	+	−	−
7	+	−	−
8	++	−	−
9	++	−	−
10	+	−	−

− = granular layer and orthokeratosis only in hinge areas of scale (Fig. 2b).

+ = intermittent areas of granular layer and orthokeratosis in non-hinge scale when inter-hinge distance was short.

+ + = continuous granular layer and orthokeratosis in non-hinge scale (Fig 2a).

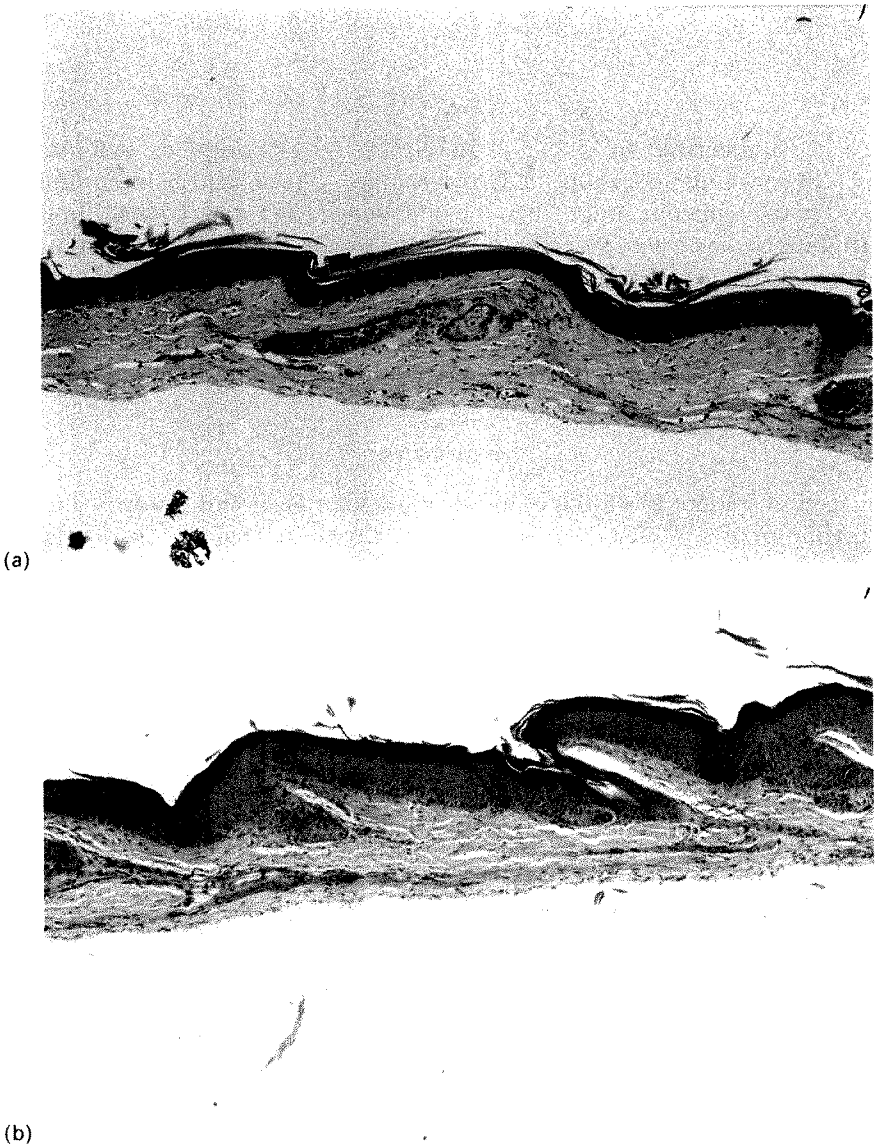

FIGURE 2. (a) Mouse tail skin showing continuous granular layer and orthokeratosis in non-hinge scale (score + +) (original × 150). (b) Mouse tail skin showing granular layer and orthokeratosis only in hinge areas of scale (score −) (original × 150).

evident in the tails of mice to which 0·2% dithranol was applied, but this was not observed after application of white soft paraffin alone or after the action of rubbing. An irritant effect was only observed in mice treated with 0·2% dithranol ointment. Dose response curves to differing concentrations of dithranol to determine a therapeutically effective concentration were not performed, but the possibility that the mouse-tail test could be used in this way deserves exploration. The effects of dithranol on proliferation indices in this model would also be of interest. In contrast to the results of Wrench and Britten[5] who used a 10% dithranol ointment, the use of an 0·2% therapeutic concentration of dithranol did cause reversion of parakeratotic to orthokeratotic scale. The mouse-tail test merits further examination in dose response studies in the evaluation of putative anti-psoriatic agents.

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Benzocaine—an unsatisfactory indicator of topical local anaesthetic sensitization for the U.K.

M.H.BECK AND ANN HOLDEN

Manchester Skin Hospital, Quay Street, Manchester M3 3HL, U.K.

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SUMMARY

Three thousand consecutive patients undergoing patch tests were tested with a mix of benzocaine, amethocaine hydrochloride and cinchocaine hydrochloride. Eighty-four subjects (2·8%) were found to be allergic to this mix. Forty of these were patch tested with the constituents of the mix: 21 (52·5%) patients were sensitive to amethocaine hydrochloride or cinchocaine hydrochloride or both, but not to benzocaine. We recommend that a non-irritant 'caine mix' containing benzocaine, cinchocaine and amethocaine should be included in the standard patch test series for the U.K.

It is usual practice for all patients to be tested with a standard series of common contact allergens when attending a clinic for contact dermatitis investigation. The International Contact Dermatitis Research Group (ICDRG) has been most helpful in recommending allergens which should be included in such a series. In 1985 they advized several changes to the content of the standard series. Included in its recommendations was the introduction of benzocaine as an indicator of contact sensitization to local anaesthetic materials. Our own knowledge of the range of different topical anaesthetic substances used in the U.K. and previous experience of contact sensitization to other local anaesthetics, in particular amethocaine hydrochloride and cinchocaine hydrochloride led us to question whether benzocaine by itself was appropriate for standard testing in the U.K. We therefore, undertook a retrospective analysis of our findings in 3000 consecutive patients patch tested with our standard series, which differed from the ICDRG recommendations by including a caine mix (5% benzocaine, 1% cinchocaine hydrochloride, 1% amethocaine hydrochloride in petrolatum) in place of benzocaine. It had been our usual practice, where feasible, to patch test subjects who had reacted to the mix with its individual constituents, and hence we have been able to analyse the pattern of sensitization caused by the three local anaesthetics.

Correspondence: Dr M.H.Beck.

METHODS

Results from three thousand consecutive patients who had been patch tested in our contact dermatitis investigation unit were studied. The patients had all been seen and assessed in a general dermatology out-patient clinic prior to referral to the unit, and they were sent to us because patch testing was felt to be an appropriate investigation for their particular skin problem. A further history and examination was undertaken in the unit immediately prior to patch testing.

Patch tests to the standard series were performed using Finn Chambers®, and were read in the standard way at 48 and 96 h.[1] All reactions scored + or greater were interpreted as being either allergic or irritant and the relevance of each reaction to the patient was assessed. Where feasible, the individual ingredients were then tested in those patients with an allergic reaction to caine mix, using the following concentrations in petrolatum: benzocaine 5%, cinchocaine hydrochloride 1%, amethocaine hydrochloride 1%. The reactions were assessed as before.

RESULTS

Eighty-four of the 3000 patients tested had allergic patch test reactions to caine mix. In 16 (19%) it was thought to have been of past relevance only. In 49 (58·4%) of the patients, the reaction was thought to be of relevance to their presenting condition. The common primary sites of involvement in the 49 patients with relevant allergic reactions were the leg (29%), anogenital region (27%), hand (16%) and face (16%). Twenty per cent of the 49 patients had stasis eczema or stasis ulcers or both.

A full analysis of the individual reactions to the ingredients of caine mix in the 40 patients tested is presented in Tables 1 and 2. More than half (53%) of the patients tested were not allergic to benzocaine, but were sensitive to amethocaine hydrochloride or cinchocaine hydrochloride or both.

TABLE 1. Results of patch testing 40 patients with the ingredients of caine mix

	Patients having an allergic reaction	
	No.	%
Benzocaine alone	16	40
Amethocaine alone	10	25
Cinchocaine alone	8	20
Benzocaine and amethocaine	2	5
Benzocaine and cinchocaine	0	0
Amethocaine and cinchocaine	3	7·5
Benzocaine, amethocaine and cinchocaine	1	2·5

TABLE 2. Total numbers of allergic reactions derived from data in Table 1

	Patients having an allergic reaction	
	No.	%
Benzocaine	19	47·5
Amethocaine	16	40
Cinchocaine	12	30
Amethocaine or zinchocaine, or both	21	52·5

DISCUSSION

Local anaesthetics can be divided into four main groups according to their structure.[2] The benzoic acid derivatives, particularly the esters of *p*-aminobenzoic acid such as amethocaine and benzocaine are well known sensitizers. Cinchocaine is a quinoline derivative and is also well recognized as a sensitiser in the U.K.[3] On the other hand, anilide or amide derivatives such as lignocaine rarely sensitize. Finally, there is a miscellaneous group. A return of 2·8% allergic patch test reactions with relevance in more than 75% of cases past or present is sufficient to justify the presence of caine mix in our standard series of allergens. It is the breakdown of the figures which is of interest, as this clearly indicates that in the U.K. the use of benzocaine as an indicator of local anaesthetic sensitization would miss more than half of those patients who were allergic to topical local anaesthetics.

Mixes can give problems both through irritancy and false negative reactions. The caine mix supplies to us by Trolab® and later by Hermal® rarely gave false positive irritant reactions and the concentrations of the ingredients are identical to those recommended when we use them individually.

The decision to choose benzocaine for the standard series was presumably taken as a reflection of its usage in topical medicaments in many countries. The incidence of sensitization varies from centre to centre, for instance in one European study nearly 10% of subjects tested in Germany, where benzocaine is a popular remedy, were sensitive to this compound, compared with 1% in Denmark.[4] Cronin[2] points out that in the U.K. benzocaine is found in relatively few preparations and that the majority of topical local anaesthetic preparations are used for anogenital pruritus and haemorrhoids. These medicaments generally contain amethocaine or cinchocaine or both, rather than benzocaine. Indeed, anogenital symptoms were the primary reason for referral in 26·5% of our allergic subjects. Nevertheless, the hands, face and legs were also common primary sites in our series, reflecting the usage of these preparations on dermatoses at a variety of sites. In particular, stasis related dermatoses were a common predisposing cause of sensitization in our series. Of significance, in our view, is the fact that many local anaesthetic preparations are available over the counter in the U.K. Benzocaine may be sufficient for patch testing in those countries where there is widespread usage of benzocaine and little use of other topical local anaesthetics, but it is insufficient for the needs of the U.K., and perhaps other countries where there may be a similar situation. The lesson of this study is clear. A non-irritant caine mix containing benzocaine, cinchocaine and amethocaine should be recommended for standard series patch testing in the U.K.

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Cyproterone acetate and two doses of oestrogen in female acne; a double-blind comparison

G.B.COLVER, P.S.MORTIMER AND R.P.R.DAWBER

Department of Dermatology, The Slade Hospital, Headington, Oxford, U.K.

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SUMMARY

Cyproterone acetate was used in combination with either 35 or 50 μg ethinyl oestradiol in a double-blind comparison of their effects on acne in females. There were no significant differences between the two groups in the degree of improvement in acne as assessed objectively by the investigators, or subjectively by the patients using a visual analogue scale. Oestrogen related side-effects were significantly lower in the 35 μg group during the course of the 9-month study.

The combination of cyproterone acetate (CPA) and ethinyl oestradiol is useful in the treatment of female acne. Doses of CPA ranging from 2 mg[1] to 100 mg[2] have been found to be effective. The dosage of ethinyl oestradiol in these studies was 50 μg daily and this alone can reduce sebum excretion rate and increase sex hormone binding globulin, both of which lead to an improvement in acne.[3] A recent study,[4] however, showed that the addition of CPA to 50 μg oestrogen significantly enhanced the effect and that this combination was more effective than standard oestrogen and progestogen contraceptive pills. The investigators also found that 2 mg and 50 mg CPA gave similar results. The aim of the present study was to ascertain whether the beneficial effect of 2 mg CPA would persist when the oestrogen content was lowered to 35 μg, a dose which has little effect on sebum excretion rate.[3] We carried out a double-blind trial to compare the safety and efficacy of 2 mg CPA/50 μg ethinyl oestradiol (D50) with 2 mg CPA/35 μg ethinyl oestradiol (D35) in female acne.

METHODS

Ninety-six females aged between 16 and 35 years entered the study, 50 receiving D35 and 46 receiving D50. Measurements were made of weight, height, and blood pressure, and details recorded of the menstrual cycle and history of acne, seborrhoea and hirsutism. These were recorded for the last untreated cycle (baseline) and for each of the nine cycles during which

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Correspondence: Dr G.B. Colver, University Department of Dermatology, The Royal Infirmary, Edinburgh, EH3 9YW, U.K.

therapy was taken. Only patients who had ceased any previous anti-acne therapy (oral and topical) at least 3 months earlier, were admitted to the study. Side effects were recorded both when reported spontaneously and in response to direct enquiry.

Acne was assessed on the face, chest and back separately and was graded as absent, mild or severe. All assessments were made by one observer (G.B.C.). For the purpose of the study the following grading system was adopted. Whiteheads and blackheads: 0–4 acne absent, 5–19 mild, 20 or more, severe; papules and superficial pustules: 0–4 acne absent, 5–19 mild, 20 or more, severe; nodules and deep pustules: 0–2 acne absent, 3–6 mild, 7 or more, severe.

The patients rated their acne on a 10 cm visual analogue scale from 'worst ever' to 'completely healed', before, during and after the study. Seventy-five patients (40 in the D35 and 35 in the D50 group) completed the visual analogue scales.

Comparisons between treatment groups were made using either Student's *t*-test, chi-square tests or Fischer's exact probability test. The significance of changes within the treatment groups were assessed by paired *t*-tests or McNemar's test, or, with small samples, the binomial test.

RESULTS

The two groups were well matched for age, weight, height, smoking habits, alcohol consumption and blood pressure. Data were available on 78 patients at the end of the study—40 in the D35 group and 38 in the D50 group. The severity of acne in the two groups before treatment and after 2, 3, 6 and 9, months of therapy are shown in Figure 1. The number of cases of severe facial acne initially was greater in the D35 group ($P<0{\cdot}05$). Acne was present in all patients at the last untreated cycle but was only present in 17 of 40 in the D35 group and 14 of 38 in the D50 group at the final assessment. The changes from baseline in the number of patients suffering from acne were statistically significant in both treatment groups at all assessments after the third cycle. After the ninth cycle $P<0{\cdot}001$ in both groups for overall acne severity and when acne on the face, chest and back were assessed separately (McNemar's test). There was no difference between the two groups at the end of the trial with regard to acne severity.

The visual analogue scale scores in the two groups did not differ significantly at the initial assessment (*t*-test). The mean changes from baseline were the same for both groups at the final assessment (Table 1). Acne improved in 39 of 40 patients in the D35 group and in 32 of 35 in the D50 group during the study period ($P<0{\cdot}001$, sign test).

TABLE 1. Visual analogue scale scores of acne severity before and after treatment. Values are means ± SD

	Score (cm)*		
	Pre-treatment	After treatment	Change in score (cm)
D35 (*n*=40)	3·3±2·2	7·9±1·6	4·5±2·4
D50 (*n*=35)	3·4±1·9	7·9±1·6	4·5±2·7

* An increase in score indicates an improvement in acne.

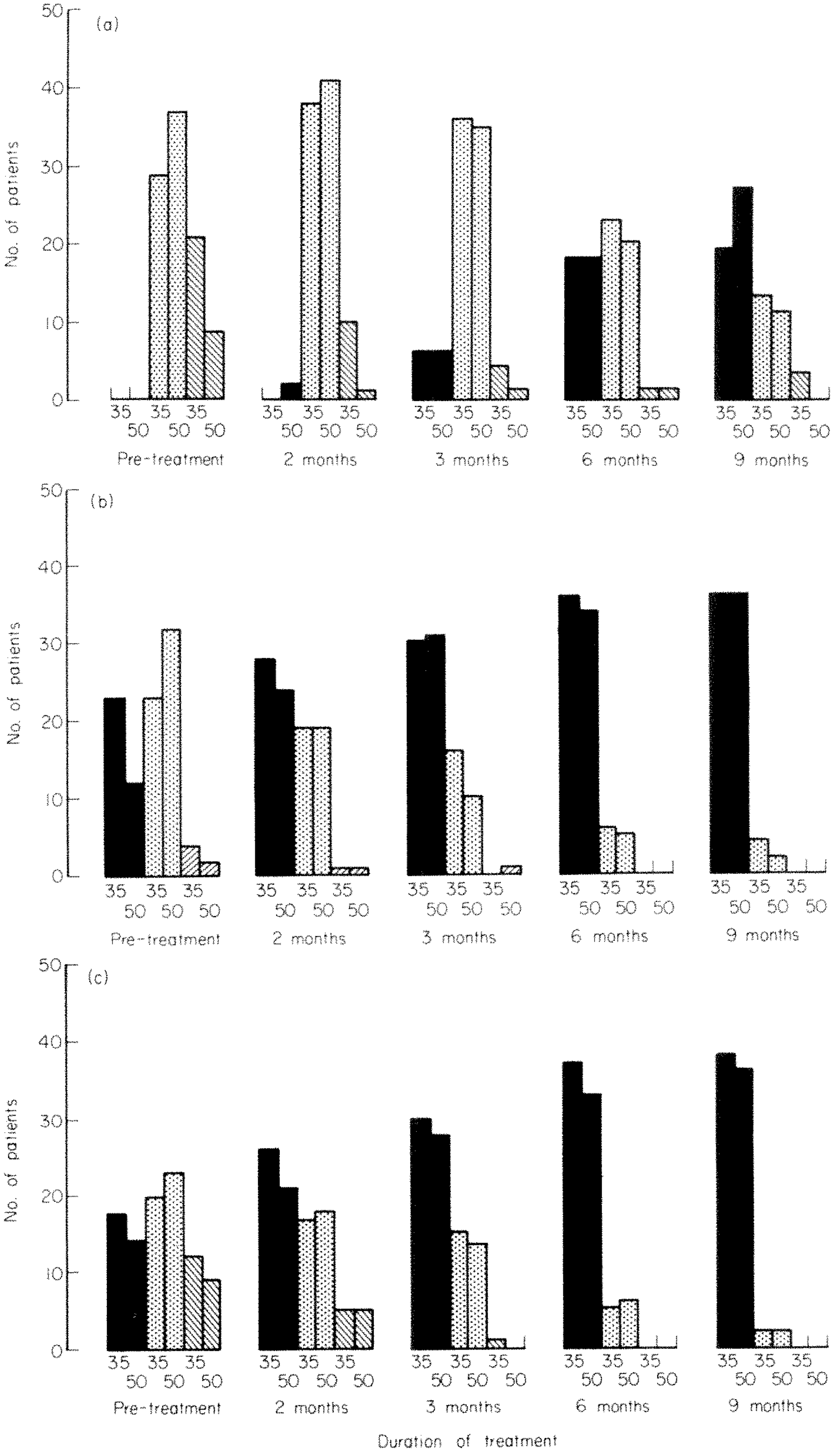

FIGURE 1. Severity of acne on (a) the face, (b) the chest and (c) the back in patients treated with D35 or D50. ■: no acne; ▨: mild acne ▧: severe acne.

Systolic and diastolic blood pressure fell slightly in both groups over 9 months. Body weight fell, but not significantly in the D35 group over 9 months and increased slightly in the D50 group but this change was only significant at the end of the first cycle. Hirsutism was objectively unchanged, but subjectively it was reduced significantly in both groups ($P < 0{\cdot}001$, McNemar's test). Cycle length, number of days of bleeding, assessment of flow and dysmenorrhoea showed no difference between the two groups. Side effects were generally mild. The most important were those usually held to be oestrogen related i.e. breast tension, headache, nausea, migraine and weight gain. The number reporting side effects was greater in the D50 group ($P < 0{\cdot}01$). Three patients withdrew because of these side effects, two in the D50 group and one in the D35 group. A further 15 subjects withdrew from the study (10 D35 and 6 D50), three because of lack of efficacy (1 D50 and 2 D35), two because of depression, one because of heavy bleeding and the others for reasons unrelated to the trial.

The design of the study and the number of patients were such that with $\alpha = 5\%$, and a success rate of 60% with Diane 50, the study had a 66% power of detecting a 20% difference if one really exists. This is the smallest difference that would be clinically significant.

DISCUSSION

The D50 preparation has been shown to be an effective treatment for acne.[1] One trial of 3 months, however, did not show an improvement.[5] However, the 3-month period used was probably too short and Wojnarowska *et al.*[6] demonstrated that 4–6 months therapy was needed to demonstrate a significant effect. 50 μg ethinyl oestradiol alone can reduce the sebum excretion rate[3] and may contribute to the efficacy of the D50 preparation. Oestrogen related side effects may be prominent at this dose, so that lowering the dose without interfering with the anti-acne effect is desirable. The present study shows that 2 mg CPA is an effective therapy when combined with 35 μg ethinyl oestradiol. Both treatment groups showed the same highly significant changes from baseline. The main effect of CPA appears to be sebostasis which is maximal at much higher doses (100 mg). At a dose of 2 mg this effect is very small. However, the present trial, and that of Miller *et al.*,[4] shows that 2 mg CPA has a clinical effect on acne, which is additional to the effect of the oestrogen in the combined preparation.

There are more accurate grading systems for acne then the one we used. It is true that our system would show no change of grade if, for example, the number of pustules on an individual's face was reduced from 9 to 5. However this was a comparative trial and showed a similar number of patients moving from the acne group to the no acne group.

Considering the size of the study group and the number of assessments required, there was a very good rate of attendance in both groups. This appeared to be due to the paucity of side effects and to patient satisfaction with therapy, resulting particularly from an early reduction in seborrhoea in both groups as assessed by the patients.

The D50 preparation (Diane) is available on prescription and may only be prescribed when the primary indication is acne. The preparation containing the lower dose of ethinyl oestradiol (35 μg) appears to offer advantages since efficacy remains unaltered whilst side effects, mostly oestrogenic in nature, are reduced.

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Hydroa vacciniforme: a review of ten cases

T.S. SONNEX AND J.L.M. HAWK

Department of Photobiology, Institute of Dermatology, United Medical and Dental Schools of Guy's and St Thomas's Hospitals, London U.K.

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SUMMARY

Ten patients with hydroa vacciniforme are reported. The condition appears to be a distinct clinical and histological entity. Haematological, biochemical, immunological, bacteriological and viral investigations were negative. Three patients demonstrated low minimal erythema doses (MEDs) following monochromatic ultraviolet (UVA) irradiation of back skin; such UVA sensitivity may be a feature of hydroa vacciniforme. Treatment was unsatisfactory, although spontaneous improvement in the condition tended to occur and regular application of sunscreens with high protection factors against both UVA and UVB was helpful.

Hydroa vacciniforme (HV) is a rare, debilitating photodermatosis of children, with distinctive clinical and histological features but unknown pathogenesis. At present there is no satisfactory treatment. Previous reports in which erythropoietic protoporphyria (EPP) has been excluded have been sporadic, describing only one or two patients each. We report here 10 patients who have been investigated and followed up in our department in the last 25 years.

METHODS

All 10 HV patients seen in the Department of Photobiology at the Institute of Dermatology since 1960 are reported. Nine were interviewed with their parents, one having been lost to follow-up after 6 years at the age of 17.

Laboratory investigations

Blood haemoglobin concentration, total and differential white cell count, erythrocyte sedimentation rate, serum urea and electrolyte concentrations, liver function, 24-hour urinary amino acids, antinuclear factor titre, viral content of early vesicles, blood viral antibody titres, stool viral content, DNA repair and urinary, faecal and erythrocyte porphyrin levels were measured.

Correspondence: Dr T. Sonnex, Institute of Dermatology, St John's Hospital, Lisle Street, Leicester Square, London WC2H 7BJ, U.K.

Histology

Lesional biopsies taken during the period of vesiculation were processed routinely and stained with haematoxylin and eosin.

Immunofluorescence

A lesional biopsy from one patient was prepared for direct immunofluorescent microscopy using routine methods.

Irradiation monochromator skin tests

All patients were irradiated on the back skin with a series of graduated doses at a range of wavelengths from 300 to 600 nm using a 2000 W xenon arc monochromator (model 498/01, F4 grating, Applied Photophysics Ltd, London U.K.).

RESULTS

The patients were all caucasian; two were male and eight female. Seven came from villages or rural areas.

The patients' skin types and hair and eye colours are summarized in Table 1.

Age of onset ranged from a few months to 5 years in the girls and was 9 and 11 years respectively in the two boys. The rash first appeared in April in six patients, in May in two and in June in two. In six of the girls the rash was still occurring at the time of interview, having improved in two, and had resolved during adolescence in two. The two boys were still affected in adolescence, one then being lost to follow-up (Fig. 1).

The eight girls were affected from March to October each year, seven relapsing in bright winter sunshine, particularly in the presence of fallen snow. The two boys were affected from May to September. All sun-exposed areas were affected, particularly the cheeks, the bridge of the nose, the V of the neck, the dorsa of the hands and the hair parting. The limbs and trunk were

TABLE 1. Skin type, and hair and eye colour in 10 patients with hydroa vacciniforme

		No. of patients
Skin type	I (always burn never tan)	3
	II (usually burn rarely tan)	6
	III (rarely burn usually tan)	1
	IV (never burn always tan)	0
Hair colour	Blonde	2
	Fair	2
	Light brown	4
	Dark brown	2
Eye colour	Blue	5
	Green	1
	Brown	2
	Hazel	2

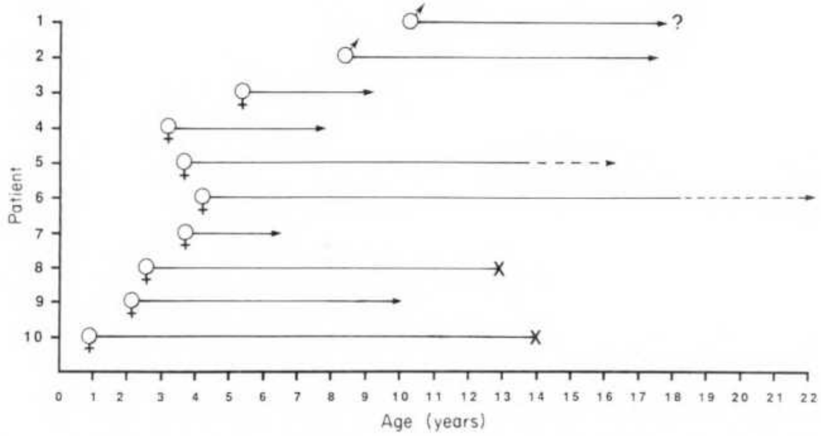

FIGURE 1. Age of onset (in males, ♂, and females, ♀) and course of the disease in 10 patients with hydroa vacciniforme. X = resolved; → = persisting; - - → = improving; ? = lost to follow-up.

also affected if exposed. Clothing gave effective protection, unless it was of light, loose weave fabric.

There were five distinct stages in evolution of the lesions. Fifteen min to 24 h after less than 1 h exposure in seven patients and after 2-6 h in three patients the sun-exposed skin became itchy and erythematous, sometimes with stinging or swelling. After a further 1 to 24 h red, pink or purple, often tender, papules appeared on the erythematous areas, often with a severe burning sensation. Five hours to 3 days later the papules became vesicular, initially painful and tense, then often umbilicated and sometimes haemorrhagic (Fig. 2). They then crusted, scabbed and eventually became pain-free about 2–7 days after onset. Within 1–6 weeks the detachment of the scabs left depressed, persistent pock scars (Fig. 3), occasionally with telangiectasia.

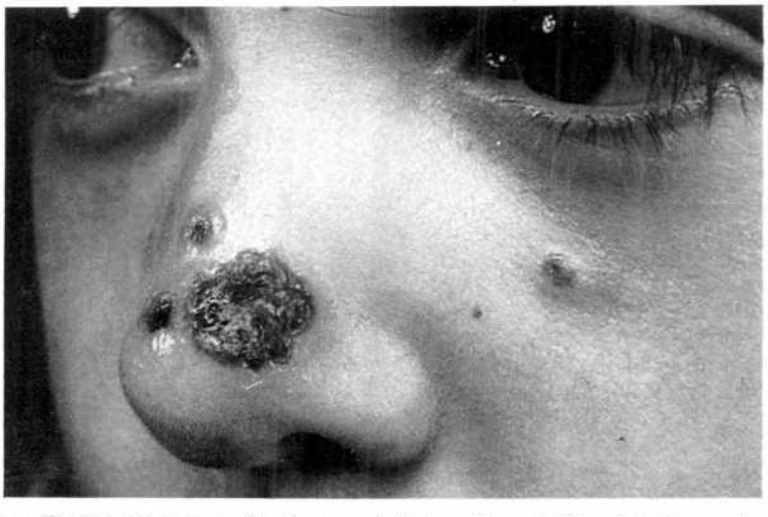

FIGURE 2. Typical appearance of hydroa vacciniforme with umbilicated vesicles and a larger discrete crusted lesion on the face.

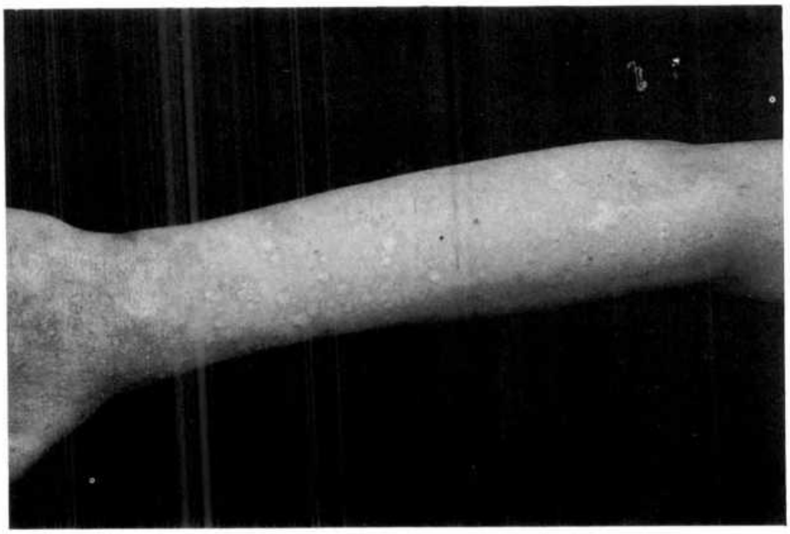

FIGURE 3. Persistent discrete scarring on the arm.

Concurrent malaise, fever and generalized aching occurred in two patients, one with redness and watering of the eyes and one with headaches. Hair, nails and mucous membranes were normal in all patients.

Laboratory investigations

Results of all laboratory investigations were normal in all patients.

Histology

All four biopsies showed early focal reticulate keratinocyte degeneration, spongiosis and a perivascular mononuclear cell infiltrate in the underlying dermis (Fig. 4). Older lesions had focal epidermal necrosis and ulceration, marked papillary oedema and areas of upper dermal necrosis (Fig. 5) with a deep and lateral mixed perivascular infiltrate, one specimen showing vessel thrombosis and erythrocyte extravasation.

Immunofluorescence

Direct immunofluorescent microscopy in one specimen showed granular C_3 and fibrin deposits below the basement membrane zone and clumps within some dermal papillae. Studies with immunoglobulins A, G and M were negative.

Irradiation monochromator skin tests

The results are shown in Table 2. All patients reacted normally to UVB (300 and 307·5 nm) and seven to UVA (320, 340, 360, 380 and 400 nm). The three girls most severely affected and least responsive to treatment had low MEDs to monochromatic UVA (340, 360 and 380 nm and 340 nm, respectively).

Response to treatment

Responses to treatment are listed in Table 2. High protection factor sunscreens giving UVB and

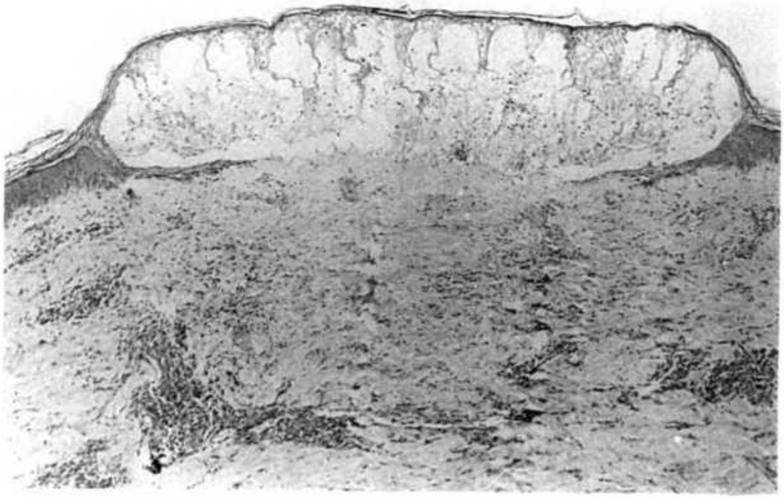

FIGURE 4. Histology of an early HV lesion with focal reticulate degeneration of keratinocytes, spongiosis, vesiculation and a perivascular mononuclear cell infiltrate in the dermis.

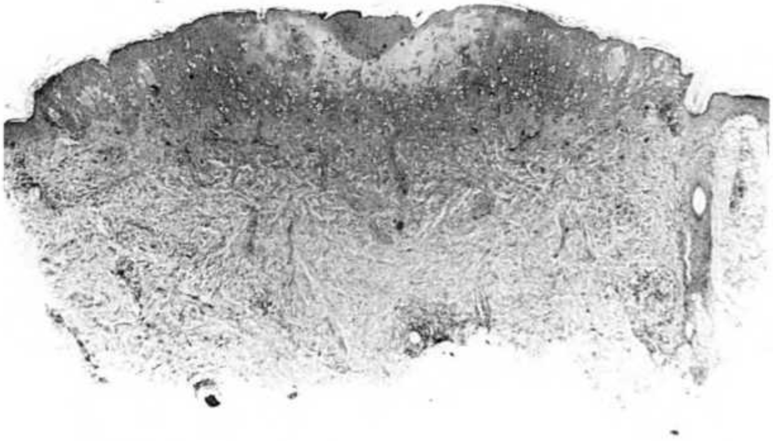

FIGURE 5. Histology of an older HV lesion with focal necrosis of the epidermis and underlying dermis. A mixed perivascular infiltrate is present in the adjacent dermis.

some UVA protection (Copper-tone Supershade 15®, RoC Factor 10®) applied regularly during sun exposure in six patients apparently enabled normal activities with minimal rash; in two others rash severity was reduced. Treatment for 2 months with chloroquine, 100–125 mg daily, apparently limited the eruption to occasional lesions in two patients, with complete relapse after stopping the drug in one case. Two patients improved with low-dose thrice weekly prophylactic UVB therapy for as long as it was continued. Low-dose prophylactic psoralen photochemother-

TABLE 2. Results of monochromator skin testing and resposes to treatment

Patient no.	Monochromator skin testing. Wavelengths at which MED abnormally low	Responses to Sunscreen	Responses to Other treatment
1	MEDs normal	+	None given
2	MEDs normal	+	None given
3	340–360 nm	±	Hydroxychloroquine, mepacrine and β-carotene ineffective
4	340–380 nm	Ineffective	Chloroquine +, UVB +, thalidomide ineffective
5	MEDs normal	Ineffective	UVB+
6	MEDs normal	+	Hydroxychloroquine ineffective
7	MEDs normal	+	None given
8	MEDs normal	±	Mepacrine ineffective
9	340 nm	+	β-carotene ineffective, PUVA precipitated HV
10	MEDs normal	+	Chloroquine +

+: enabled patient to lead a normal life during the summer months
±: reduced severity of rash

apy (PUVA) precipitated the eruption in one. Mepacrine 200 mg bd, β-carotene 100–200 mg daily for some weeks and hydroxychloroquine 100 mg nocte in one patient were ineffective.

DISCUSSION

Hydroa vacciniforme, described by Bazin[1] was at first infrequently diagnosed because of terminological confusion and uncertainty concerning the role of porphyrin metabolism in its pathogenesis.[2,3,4] Later it was accepted as distinct, being described in a review of 29 cases as demonstrating light-induced vesicles and crusts leading to scarring in spite of normal porphyrin biochemistry, although EPP was not specifically excluded.[5] This description applied to all our patients, in whom we did exclude EPP, upholding the view that HV is a separate entity. Lesions appeared in summer and occasionally in winter on exposed areas, initially itchy erythema progressing to painful, stinging papules within hours of often minimal sun exposure. Vesicles developed hours later followed by scabbed, crusted lesions and finally, pock-like scars. Onset in childhood was typical, although a 1-year-old infant was affected, and lesion severity diminished over several years in three patients, and resolved suddenly over 1 year in one. Lesions continued into adulthood in only one patient. There have been previous reports of this in rare cases.[5,6] A female preponderance of 8:2 was found in our patients, similar to the 10:6 ratio reported by Halasz *et al.*,[6] although McGrae & Perry[5] reported a ratio of 19 males: nine females. We did not find a family history of HV, as has been reported on one occasion in three siblings.[7]

Fair, easily sunburned skins were most common in our patients; Halasz *et al.*[6] also noted a poor tanning response in their patient. It may be that increased UV sensitivity in HV is partly related to some impairment of physiological protection, although this does not explain the focal nature of the eruption.

Histology in our patients showed the previously described characteristic features, in addition to vessel thrombosis in one patient, which may possibly contribute to the focal nature of the necrosis.

All our patients had normal urinary and faecal as well as erythrocyte porphyrin levels, ruling out all forms of porphyria. Xeroderma pigmentosum was excluded by normal results in DNA repair studies and metabolic disorders previously reported to be associated with HV, namely aminoaciduria[8] and Hartnup's Disease,[9] were excluded by negative findings on urinary amino acid screening. We found no abnormalities in systems other than the skin to account for the non-specific malaise and generalised aching in two patients during HV attacks. Tests for virus infection were negative.

There is strong evidence of a causal relationship between HV and UV exposure from the development of the lesions and their distribution. However, the wavelengths involved and the pathogenetic mechanisms remain uncertain. The cutaneous response to monochromatic irradiation in three of our patients suggested that UVA sensitivity may be important. Previous reports of skin responses to artificial irradiation in HV patients, summarized in Table 3, support this finding. Repetitive broad spectrum UVA irradiation has been shown to induce skin lesions indistinguishable from the natural eruption.[6,10,11] Furthermore, clinical improvement during therapy with hydroxychloroquine has been associated with an increase in the UVA MED and in the number and magnitude of UVA doses required to reproduce clinical lesions.[10]. UVB sensitivity in HV has also been suggested,[12–14] although not as convincingly as UVA sensitivity, and further work is required to clarify the exact action spectrum.

Treatment of HV is unsatisfactory. Recent reports (Table 3) have suggested that antimalarials, β-carotene and UVB can be helpful, but in our patients the most successful treatment was regular application of high protection factor sunscreens against UVA and UVB, while chloroquine and UVB phototherapy each appeared helpful in two patients. However, β-carotene, thalidomide, hydroxychloroquine and mepacrine were ineffective. PUVA precipi-

TABLE 3. Recently published data on MEDs and responses to treatment in HV patients

Source	Sex	Age at onset (years)	Single dose phototesting	Attempted reproduction of HV lesions	Response to treatment
Schiff and Jillson[14]	M	9	Normal MED UVB	8 × MED UVB: erythema, papules and vesicles	Chloroquine-effective
Ramsay[13] (Patient no. 6 in our series)	F	5	Normal MED, monochromatic UVB + UVA, abnormal papular response at 300 nm	Not done	
Bickers *et al.*[12]	M	3	Normal MED UVB	15 × MED UVB + UVA: bullous lesions, no scarring	Hydroxychloroquine—partially effective β-Carotene—effective
	F	6	Normal MED UVA and UVB	Not done	β-Carotene—effective
Jaschke and Hönigsmann[11]	M	14	Normal MED UVA and UVB	Repetitive UVA: HV lesions	β-Carotene—ineffective PUVA—effective
Goldgeier *et al.*[10]	M	12	Normal MED UVA and UVB	Repetitive UVA: HV lesions; repetitive UVB: no effect	Sunscreens, β-Carotene—ineffective Hydroxychloroquine: (200 mg/day) effective
Halasz *et al.*[6]	F	15	Normal MED UVB Reduced MED UVA	Repetitive UVA: HV lesions; repetitive UVB: no effect	Mepacrine (200 mg/day)—ineffective

tated an exacerbation, as has been reported by Halasz *et al.*,[6] although PUVA has also been reported to be effective in HV.[11] We recommend, therefore, that regular application of a high protection factor suncscreen against both UVA and UVB, combined if necessary with an antimalarial, β-carotene, UVB phototherapy or perhaps careful PUVA therapy should be tried.

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Angiosarcoma arising in an angiomatous naevus following irradiation in childhood

S.E.HANDFIELD-JONES, C.T.C.KENNEDY AND J.B.BRADFIELD*

Departments of Dermatology and * Histopathology, Bristol Royal Infirmary, Bristol BS2 8HW, U.K.

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SUMMARY

We describe a patient who developed an angiosarcoma on her face at the age of 24. The tumour arose from an angiomatous naevus that had been treated with radiotherapy when the patient was 7 months old.

Angiosarcomas are rare tumours usually occurring on the face in the elderly, or, less often, in a chronically lymphoedematous limb (Stewart-Treves syndrome). Irradiation induced angiosarcomas have been reported arising in both skin[1] and internal organs.[2]

CASE REPORT

The patient had had an angiomatous naevus on the right side of her face at birth. It was flat initially, but gradually grew in size. At 7 months of age it was causing displacement of the right eye. The patient was treated with radiotherapy without a biopsy being taken. She received three courses of cobalt 60 irradiation, divided over 6 months, to a total skin dosage of 2600 cGy. Six months later a residual nodule was treated once only with Grenz rays. A small nodule remained at the inner canthus.

In 1981 when the patient was 20 years old the nodule increased in size (Fig. 1). It was thought to be an extension of a benign angioma and was excised locally. Two years later a further recurrence was removed. At the age of 24 the malignant nature of the tumour was appreciated, and wide excision and grafting was undertaken. One year post-operatively there was no sign of local or distant recurrence.

HISTOLOGY

The first specimen in 1981 showed a vascular lesion, with spaces lined by endothelial cells. One area was more solid, but at higher magnification the cells were uniform and appeared benign (Fig. 2).

The specimen taken in 1983 was predominantly solid. The tumour cells had split up and invaded muscle bundles. At high magnification, the solid areas showed some cells with larger nuclei, but the cells lining the vascular spaces were evenly sized and flat (Fig. 3).

Correspondence: Dr S.E.Handfield-Jones

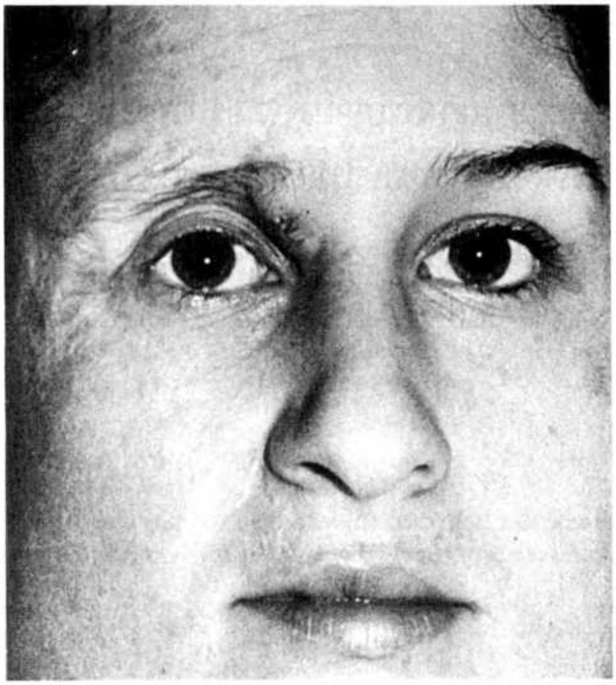

FIGURE 1. Swelling above right inner canthus. (The patient gave written permission for publication of unretouched photograph).

In 1985 the tumour showed both vascular spaces and solid areas. The cells lining spaces were haphazardly arranged and at higher magnification were plump and atypical. Solid areas were formed from pleomorphic cells with large pale nuclei and occasional mitoses (Fig. 4).

Factor VIII related antigen staining was positive both in solid tumour areas and in cells lining the vascular spaces.

DISCUSSION

Angiomatous naevi are a common finding in infants. After an initial increase in size they regress and often disappear in later childhood. Spontaneous malignant change is extremely rare.[3]

Malignant change induced by radiotherapy has been reported four times, to our knowledge, since 1950.[4–7] In all cases the naevus was irradiated in childhood and after a long latent period of betweeen 12 and 30 years, angiosarcoma was diagnosed. Radiation dosage was documented in only one case and was 1350 cGy at the skin surface. A previous study of 5000 children with haemangiomas treated by irradiation showed no excess of malignancy,[8] but the follow-up period was 20 years which may well prove to be too short. Also the initial radiation dosage of 300–600 cGy was less than that in our patient.

Angiosarcomas are aggressive tumours and those arising from irradiated naevi seem to be no exception. Treatment is often delayed because of the difficulty in diagnosis. Surgical excision has to be much wider than the visible tumour to avoid local recurrence. Radiotherapy has been used to treat local lesions, but when the tumour is radiation induced, further treatment is often contraindicated. Chemotherapy has variable results, but scarcity of cases makes a formal trial impossible.

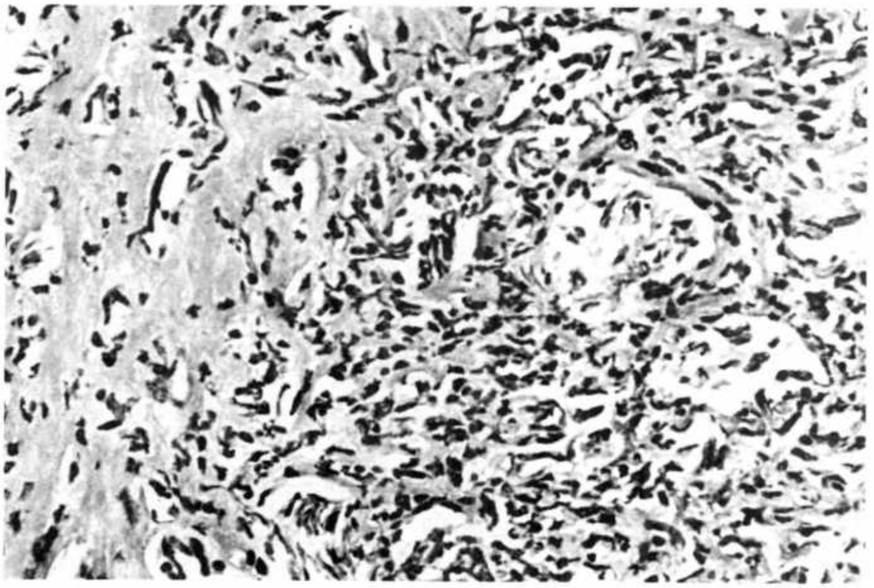

FIGURE 2. Histology in 1981. Cells are uniform and appear benign (original × 325).

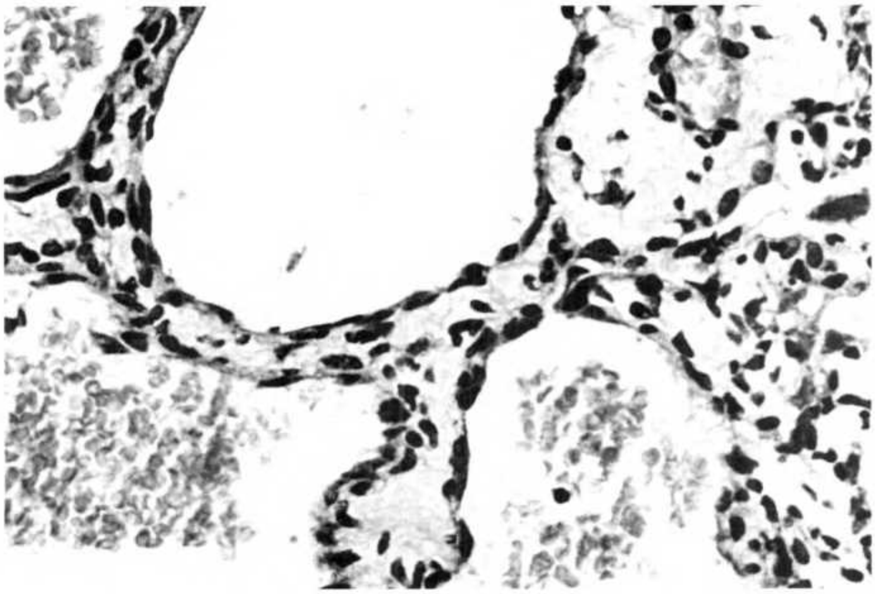

FIGURE 3. Histology in 1983. Vascular spaces lined with evenly sized flat cells (original × 325).

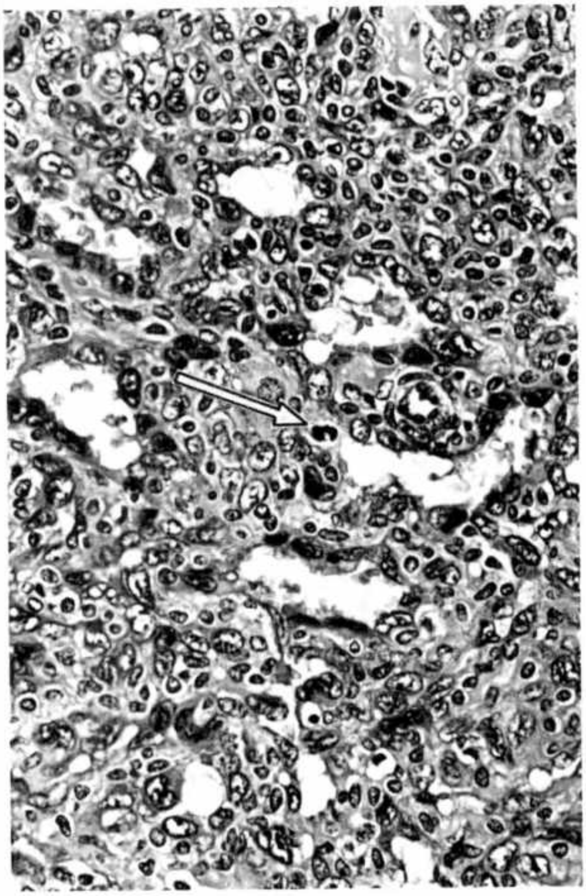

FIGURE 4. Histology in 1985. Cells lining the spaces are plump and atypical (arrow indicates mitosis) (original × 325).

Although no longer used for benign lesions in childhood, it was only 30 or 40 years ago that radiotherapy was used extensively for treatment of capillary naevi. With the long latent period between treatment and malignant change there may still be people who are at risk of developing an angiosarcoma.

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Pseudoainhum and erythropoietic protoporphyria

A.P. CHRISTOPHER, C.E.H. GRATTAN AND M.A. COWAN

Department of Dermatology, The General Hospital, Birmingham B4 6NH, U.K.

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SUMMARY

We report the development of pseudoainhum in a patient with erythropoietic protoporphyria, the first report, to our knowledge, of these two unusual conditions occurring together. An excellent cosmetic and functional result was achieved with a Z plasty.

Pseudoainhum has been reported in association with a number of hereditary and acquired disorders. It is characterized by the appearance of a constricting band around a digit or limb which may lead to spontaneous amputation. It differs from the term ainhum, in which the fifth toe is involved with eventual autoamputation, as first described by daSilva Lima in 1880[1], and which excludes congenital varieties and those secondary to any associated disease.

CASE REPORT

A 42-year-old Caucasian woman was first seen by a dermatologist at the age of 1 year with an unduly dry skin and a prematurely aged appearance. At the age of 9 a diagnosis of porphyria was suspected when she was found to have a high faecal excretion of protoporphyrin, but it was not until 20 years later that a definitive diagnosis of erythropoietic protoporphyria (EPP) was established by the finding of greatly increased levels of erythrocyte protoporphyrin (1000 μg/dl of red cells; upper limit of normal, 4·2 μg/dl). Her brother had the same condition.

The patient was treated with β-carotene from March to September each year, starting in 1973, and remained well apart from some thickening and hyperkeratosis on the dorsa and sides of her fingers up to the knuckles, and to a lesser degree on the palmar surfaces, with recurrent fissuring (Fig. 1). A liver biopsy in 1981 showed normal parenchymal architecture with no inflammation or fibrosis. A small amount of fine granular golden brown pigment was present in the hepatocytes, which fluoresced under polarized light, probably due to accumulation of protoporphyrins. However, at the age of 41 she developed a gradually increasing band of constriction around the terminal interphalangeal joint of the right index finger with swelling and pain on either side of the constriction. Increased curvature of the nail with transverse ridging was also noted (Fig.2). A full blood count and liver function tests were normal. X-rays of the hand showed some minor erosive changes on the palmar aspect of the distal interphalangeal joint below the constricting band. Swabs from the affected area grew *Staphylococcus aureus* but

Correspondence: Dr A.P. Christopher.

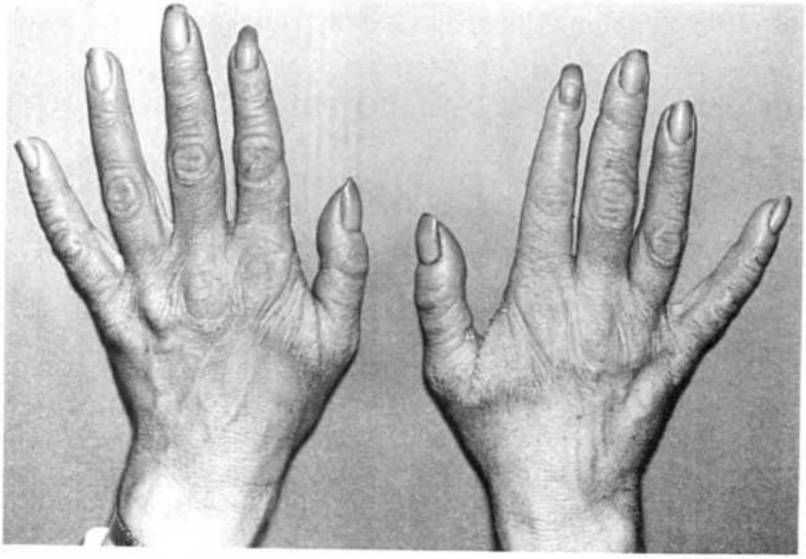

FIGURE 1. Thickening and scaling on the knuckles and backs of the fingers, with early signs of a constriction band around the terminal interphalangeal joint of the right index finger.

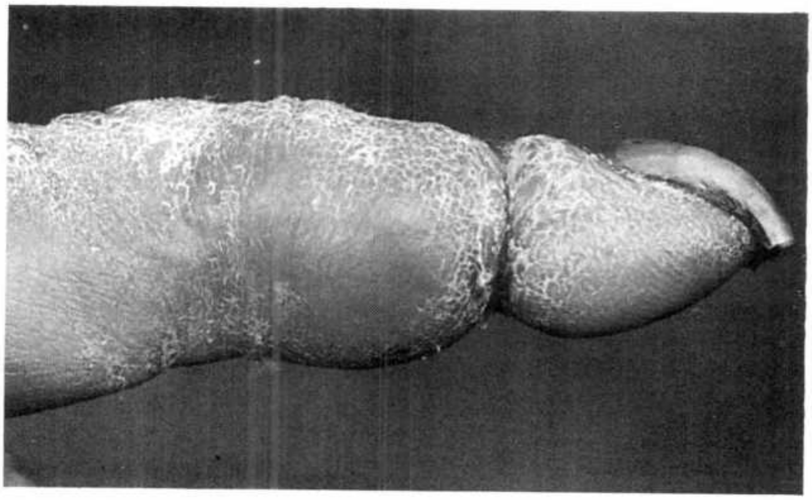

FIGURE 2. Lateral view of the right index finger showing the constriction band at the level of the terminal interphalangeal joint with considerable swelling of the digit and increased curvature of the nail.

mycology was negative. The constricting band was released by a plastic surgeon using a 'Z' plasty technique with an excellent cosmetic and functional result (Fig. 3).

DISCUSSION

Pseudoainhum and ainhum are usually grouped together since the pathogenesis, pathology and

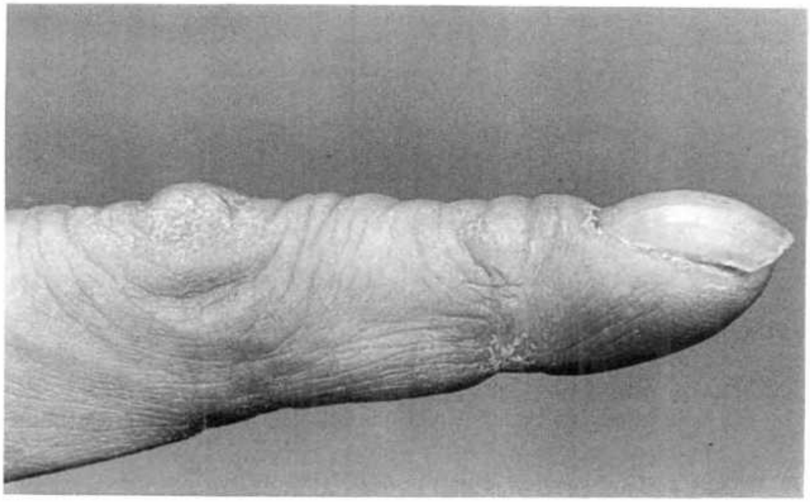

FIGURE 3. Postoperative appearance of the right index finger showing restoration of the normal contour of the finger and lessening of the nail curvature.

the end result are the same in both conditions. The disorder has been described with several hereditary and acquired dermatoses including palmoplantar keratoderma, pachyonychia congenita, Vohwinkel's disease, mal de Meleda syndrome, pityriasis rubra pilaris, fungal infection, leprosy, psoriasis and following trauma. It has also been seen in a number of systemic disorders including systemic sclerosis, Raynaud's disease, ergot poisoning, diabetes mellitus, syringomyelia, some spinal cord tumours, treponematosis, ankylostomiasis and cholera. There have been several reports of associated angiodysplasia.[2,3]

The pathological changes of digital degeneration, osteoporosis and bony erosions, with progression to autoamputation, are thought to result from strangulation of digital blood vessels and direct pressure atrophy due to fibrosis.[4] Secondary infection and osteomyelitis are frequent complications.[5] Although what exactly starts the process is not clear, an attempt has been made to explain the aetiology under three headings, infection, constitutional and mechanical. Low grade cellulitis and fibrosis of the corium, fibrogenetic tendency of the race, hyperkeratosis of the palmoplantar region and injury seem to be strong aetiological associations.[6] Chronic fissuring often occurring in hyperkeratotic skin is one of the few constant aetiological factors.[7] It is, therefore, conceivable that the remitting cutaneous symptoms of EPP, which often turn into permanent pachyderma,[8] led in our patient to repeated fissuring of the terminal interphalangeal joint which in turn facilitated secondary infection, finally progressing to pseudoainhum.

It is also important to note that early recognition of the condition and Z plasty can prevent an otherwise relentless progression to autoamputation.

ACKNOWLEDGMENT

We thank Mr J.D. Nancarrow for his surgical help.

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Malignant atrophic papulosis (Degos' disease)—Diffuse involvement of brain and bowel in an African patient

R.J.BARLOW, T.HEYL, I.W.SIMSON* AND E.J.SCHULZ

Medical University of Southern Africa, Pretoria, and *University of Pretoria, South Africa

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SUMMARY

We report pathognomonic skin lesions of malignant atrophic papulosis, accompanied by lethal central nervous system and gastrointestinal disease in a 22-year-old black man. Histopathological studies of the brain and colon showed areas of infarction and an underlying vasculitis with leukocytoclasis.

Malignant atrophic papulosis is a rare disorder, fewer than 90 cases having been reported in the literature, to our knowledge. Clinical manifestations are caused by a thrombosing endarteritis[1] which is often associated with a perivascular infiltrate of lymphocytes and histiocytes.[1–5] Typical skin lesions have been present in all the patients described.[1] In some patients systemic involvement resulted in small bowel perforations[3,7] cerebral infarction[1,8–10] or both[11]. We report a patient with malignant atrophic papulosis in whom characteristic skin changes were present as well as lethal involvement of both the central nervous system and gastrointestinal tract.

CASE REPORT

A 22-year-old black man was referred to us from a hospital in Namibia. The only information available was that he had been ill for some months, had developed weakness of the legs and had become stuporose a few days before admission.

Examination revealed a lean, apyrexial patient. There was an eruption confined to the trunk and upper arms which consisted of about 30 discrete scattered lesions. Early lesions were round or oval and faintly erythematous papules 0·5–1·0 cm in diameter. Most, however, consisted of a depressed, porcelain white scar covered by loosely attached scale and surrounded by a hyperpigmented, telangiectatic rim. Most older lesions were round, but a few were linear or

Correspondence: Professor E. J. Schulz, Division of Dermatology, (Area 553), Johannesburg Hospital and University of the Witwatersrand, Parktown, Johannesburg, 2193, South Africa.

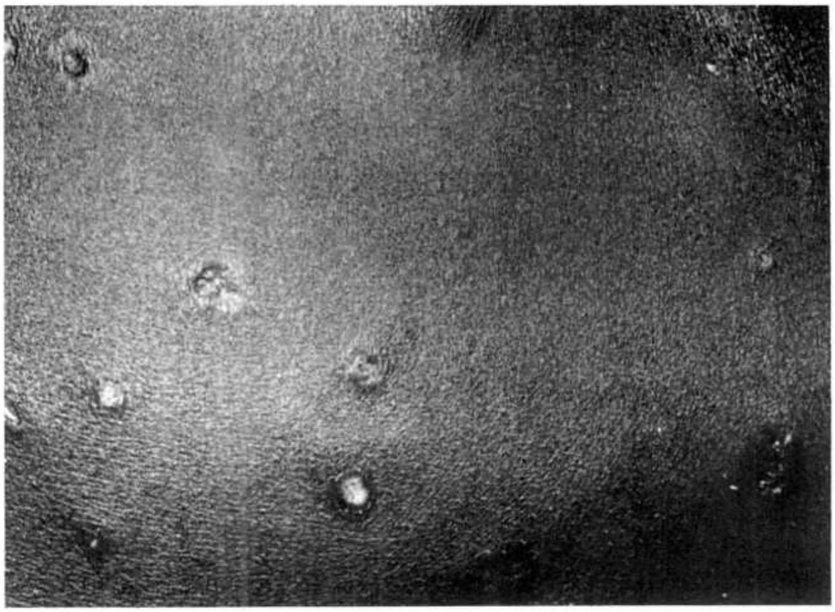

FIGURE 1. Papules and atrophic scars on the chest.

angulated (Fig. 1). A generalized shotty lymphadenopathy was present. The abdomen was flat and tender to palpation with voluntary muscle guarding. There was no rigidity, and bowel sounds were normal. Neurological examination revealed a stuporose patient with a positive jaw jerk and Hoffman's sign. There was a right facial nerve palsy. Upper motor neurone signs were present in the arms and lower motor neurone signs in the legs. Sensation was absent below the nipples and the patient was incontinent of urine. An initial computerised axial tomographic brain scan revealed a non-specific layered pattern suggestive of encephalitis.

Laboratory investigations

The following were normal or negative: full blood count and differential counts, erythrocyte sedimentation rate, blood urea and electrolytes, blood glucose, blood cultures, malaria smears, antinuclear factor and syphilis serology. Liver function tests showed a total protein level of 67 g/l (normal 60–80 g/l), serum albumin 32 g/l (normal 35–55 g/l), alanine transaminase 39 U/l (normal <23 U/l), and gamma glutamyl transferase 18 U/l (normal <40 U/l). The cerebrospinal fluid had a protein concentration of 1493 mg/100 ml (normal 15–45 mg/100 ml), lymphocytes 38/ml (normal 0–5/ml) and neutrophils 23/ml (normal 0).

Histology. A biopsy specimen from an early papule showed a normal epidermis. There was a wedge-shaped dermal infarct with peripheral capillary dilatation and a mild perivascular lymphohistiocytic infiltrate. Alcian blue staining showed no abnormal deposition of mucin in the dermis. Sections of an older lesion revealed parakeratotic scale covering an acanthotic epidermis. Under this the residue of a fibrinous exudate and a superficial hyaline scar were visible. At the periphery, there were dilated capillaries surrounded by a lymphohistiocytic infiltrate. In the oldest lesion biopsied, parakeratotic scale covered a localized area of epidermal atrophy bordered by normal epidermis.

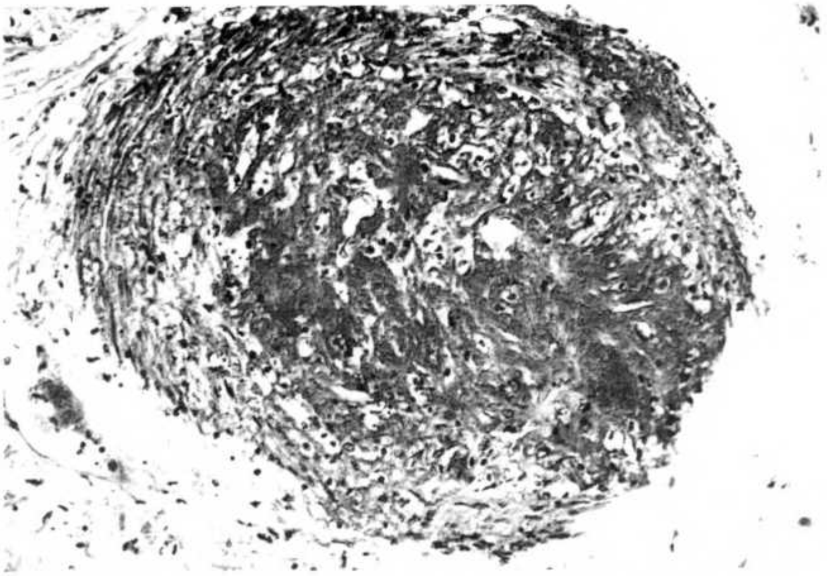

FIGURE 2. Occluding thrombus in a muscular artery, with a sparse neutrophilic infiltrate and leukocytoclasis in the vessel wall and the thrombus. (H & E; original × 252.)

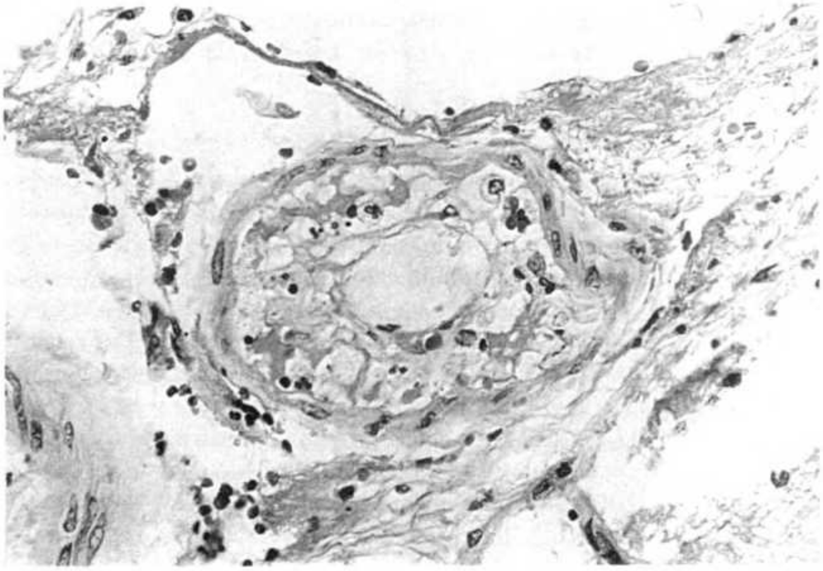

FIGURE 3. Intimal thickening in an older lesion in a small artery, showing incorporated fibrin, several neutrophils and macrophages. (H & E original × 400).

Clinical course. Three days after admission the patient was found to have a rigid abdomen with absent bowel sounds. An erect abdominal X-ray revealed air under the right hemidiaphragm and a laparotomy was performed the same day. The surgeons reported an acute peritonitis with 'necrotic plaques' on the mesentery of the small bowel. No sites of perforation were evident.

During the following few days the patient became comatose with fixed dilated pupils. A

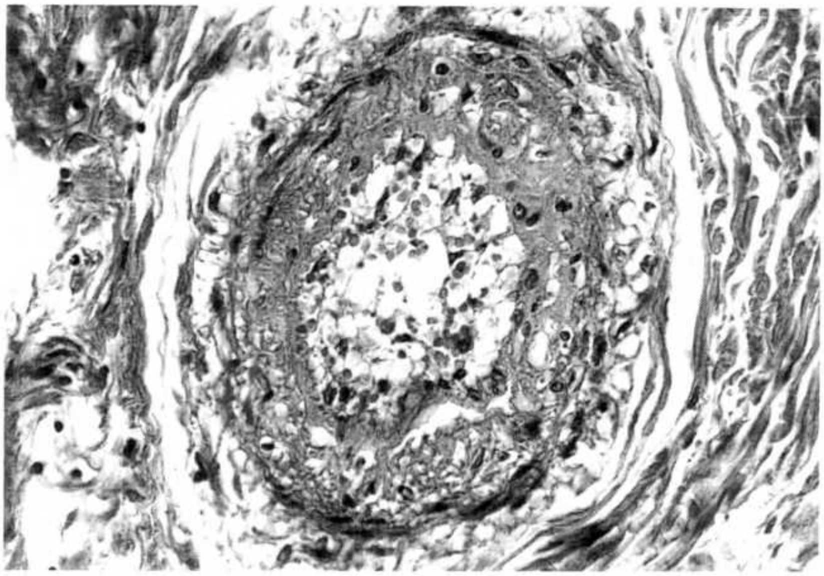

FIGURE 4. A small artery showing dense fibrosis in the deeper intimal lesion with entrapped red cells in the superficial loose connective tissue. (H & E; original × 400)

second brain scan performed 7 days after admission showed a large left sided frontoparietoccipital infarct surrounded by oedema and associated with a shift of the midline to the right. He died on the eleventh day after admission.

Autopsy findings

The typical skin lesions of malignant atrophic papulosis were present as described above.

A partly bile stained fibrinopurulent exudate in the peritoneal cavity indicated small bowel perforation. There were large and small submucosal haemorrhages in the stomach and reddish areas of ischaemia and infarction in the sigmoid colon. On histological examination the mucosa of the colon showed focal ulceration and the submucosa was oedematous. The arteries in the bowel wall showed occlusive lesions in different stages of development. The earliest lesion was a fibrin thrombus completely occluding the lumen. This was associated with a mild neutrophilic infiltrate with leukocytoclasis, involving the thrombus, the vessel wall and the perivascular connective tissue (Fig. 2). Small veins adjacent to the involved arteries were also occluded by fibrin thrombi. Older lesions showed intimal thickening in which fibrin was incorporated (Fig. 3). Dense fibrosis characterized the deeper intimal lesion, but red cells were often entrapped in the loose superficial connective tissue (Fig. 4). A sparse infiltrate of neutrophils was present even in some of the older occluding lesions (Fig. 5).

Sections of an abdominal lymph node showed no abnormalities. Microabscesses found in the liver and the adrenal glands were consistent with septicaemia.

Gross examination of the brain showed diffuse swelling of the left cerebral hemisphere with engorgement of the cortical veins and flattening of the sulci. There was a large infarct in the left frontoparietoccipital region with surrounding oedema and haemorrhage. Subfalcine herniation was present. The arteries and venules of the subarachnoid space showed acute lesions similar to those already described in the colon. Immunoglobulin deposits were not detected immunocytochemically.

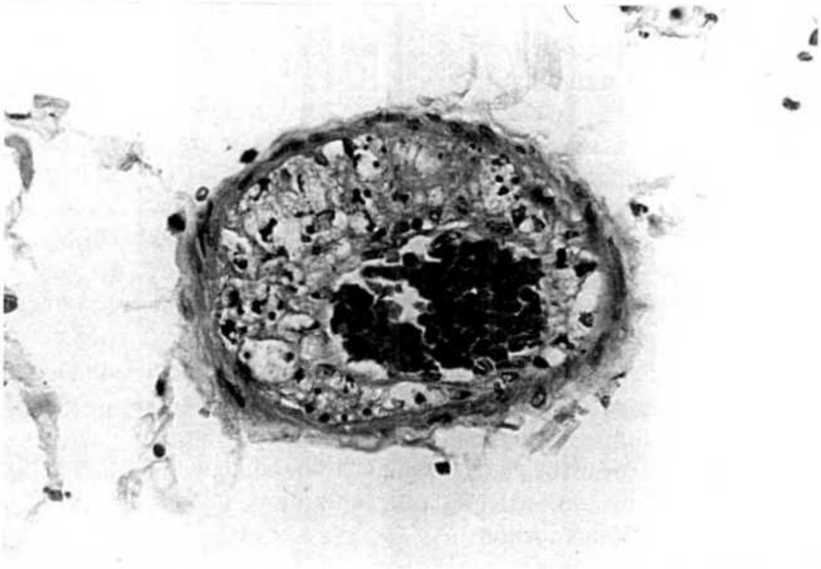

FIGURE 5. An old arterial lesion showing a sparse infiltrate of neutrophils in the connective tissue. (H&E; original × 400).

DISCUSSION

The aetiology of malignant atrophic papulosis in unknown. The outcome is usually fatal[12] although a benign and chronic variety which is limited to the skin has also been recognized.[5]

The skin eruption mainly involves the trunk and proximal extremities and usually occurs in previously healthy individuals.[3,10,13,14] Fully evolved lesions are pathognomonic[13] and are round or linear with a central porcelain white scar and a telangiectatic rim. Skin lesions may be followed within weeks or years by abdominal symptoms[3,15] which often precede fulminating peritonitis and death. In these patients, pathological findings include mucosal ulceration[7] and avascular serosal plaques.[3,6,7,11,16,17] Perforation has been observed in some cases.[3,15,18] Central nervous system involvement was reported as an unexpected post-mortem observation in two patients who had died of gastrointestinal crises.[11,19] In later reports, significant[20] or lethal[1,8,9,] neurological disease was associated with mild abdominal symptoms and signs in some patients.[21] Other organs which may be affected are the lungs[21] and pleurae,[4] the heart,[2,3,11] the gallbladder,[4] the kidney[11] and the urinary bladder.[2]

The skin lesions show characteristic pathological changes. The epidermis is atrophic and hyperkeratotic and overlies a wedge shaped infarct in the dermis.[6] An occluded vessel showing endarteritis is sometimes seen in the lower dermis or subcutis at the apex of the lesion.[3,14,16] The vascular changes of malignant atrophic papulosis begin with endothelial proliferation, swelling and fibrinoid necrosis in small dermal vessels with narrowing of the lumina, thrombosis and obstruction.[6,7] The vascular lesions and consequent tissue changes in the gastrointestinal tract, central nervous system and other organs are often remarkably similar to those described in the skin. In bowel lesions, necrosis of the mucosa[6,11] and fibrosis of the submucosa and serosa[3] result from endarteritis[2,7] and thrombosis[3,15] of small arteries. Neighbouring veins have been reported to be dilated and thrombosed.[6,15] In patients with neurological disease, the brain[1,9,20,21] cerebellum[9,21] and spinal cord[8,9,21] may be involved. Vessels which vary in size from small arterioles[9,11] to large arteries[1] and even the internal carotid may be affected by endarteritis which results in infarction.[8,11]

Degos[6,13,22,23] has repeatedly stated that inflammatory infiltrates are sparse or absent except in early lesions. However, it has recently been suggested that the important pathological change is actually a lymphocyte mediated vasculitis.[5,20] In our patient, neutrophils were present in and around vessel walls in the bowel and brain. Neutrophilic infiltrates have been described in four previous reports involving vessels in the skin. In two of these,[3,24] neutrophils formed part of a mixed infiltrate in the intima, in a third[18] they were partly broken up and were present in the wall and in the last[15] they were perivascular. Two previous reports have described neutrophilic infiltrates of vessels in organs other than the skin. In one patient neutrophils were seen in the intima of a small mesenteric artery[2] and in the other in the walls of small subarachnoid vessels.[21] It is likely that infiltration by neutrophils in our patient was a primary event. If it had been secondary to infarction, a diffuse rather than a perivascular infiltrate would have been expected. The neutrophilic infiltrate and associated leukocytoclasis seen in the small arteries in our patient are suggestive of an arteritis due to circulating immune complexes, despite our failure to demonstrate immunoglobulin deposition.

To our knowledge, only one other black patient with Degos' disease has been reported.[2] He was a young American man who also seems to have been the only other case with generalized lymphadenopathy. Post-mortem examination revealed multiple bowel perforations with gastric and rectal ulcers. This is the only other patient who, like ours, had a neutrophilic vasculitis of the intestinal tract.

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Halo dermatitis around a melanocytic naevus: Meyerson's naevus

D.S.H.NICHOLLS AND G.H.MASON*

Department of Dermatology, Auckland Hospital, Auckland and *Department of Pathology, North Shore Hospital, Auckland, New Zealand

Accepted for publication 13 July 1987

SUMMARY

A case of halo dermatitis (eczema) around a pigmented naevus is reported. This appears to be a not uncommon condition which is not described in major dermatology textbooks. The relevant literature is reviewed, and the eponym 'Meyerson's naevus' is suggested.

CASE REPORT

A 21-year-old-man presented in October 1985 with a pruritic eruption around a previously noted pigmented naevus on his right medial calf. The eruption had been present for 2 months. The patient was atopic with a past history of asthma and hayfever, but was otherwise well. No external contact factors were noted and he was on no medication. On examination, a 12 mm eczematous halo was noted around a 3 mm pigmented naevus on the right medial calf (Fig. 1). There were no other abnormalities of the skin. The lesion was excised.

Histology

There was psoriasiform hyperplasia of the epidermis with spongiosis and focal parakeratosis. Centrally, there were lentigo-like features, and benign appearing naevus cells were present in the papillary dermis (Fig. 2) associated with scattered melanophages. There was a superficial perivascular lymphohistiocytic infiltrate with moderate numbers of eosinophils (Fig. 3).

DISCUSSION

Halo dermatitis around a melanocytic naevus was first described in 1971 by Meyerson.[1] He reported two male patients with multiple pruritic papulosquamous lesions on the trunk and proximal extremities, which almost exclusively involved pigmented naevi. Histologically, these lesions showed pigmented melanocytic naevi with overlying acanthosis, spongiosis and parakeratosis, and a dermal perivascular lymphocytic infiltrate.

Krivanek *et al.*[2] described a 21-year-old man with a junctional naevus and a surrounding halo of eczema. Histology showed changes interpreted as an involuting junctional naevus with

Correspondence: Dr G.H.Mason, Department of Pathology, North Shore Hospital, Auckland 9, New Zealand.

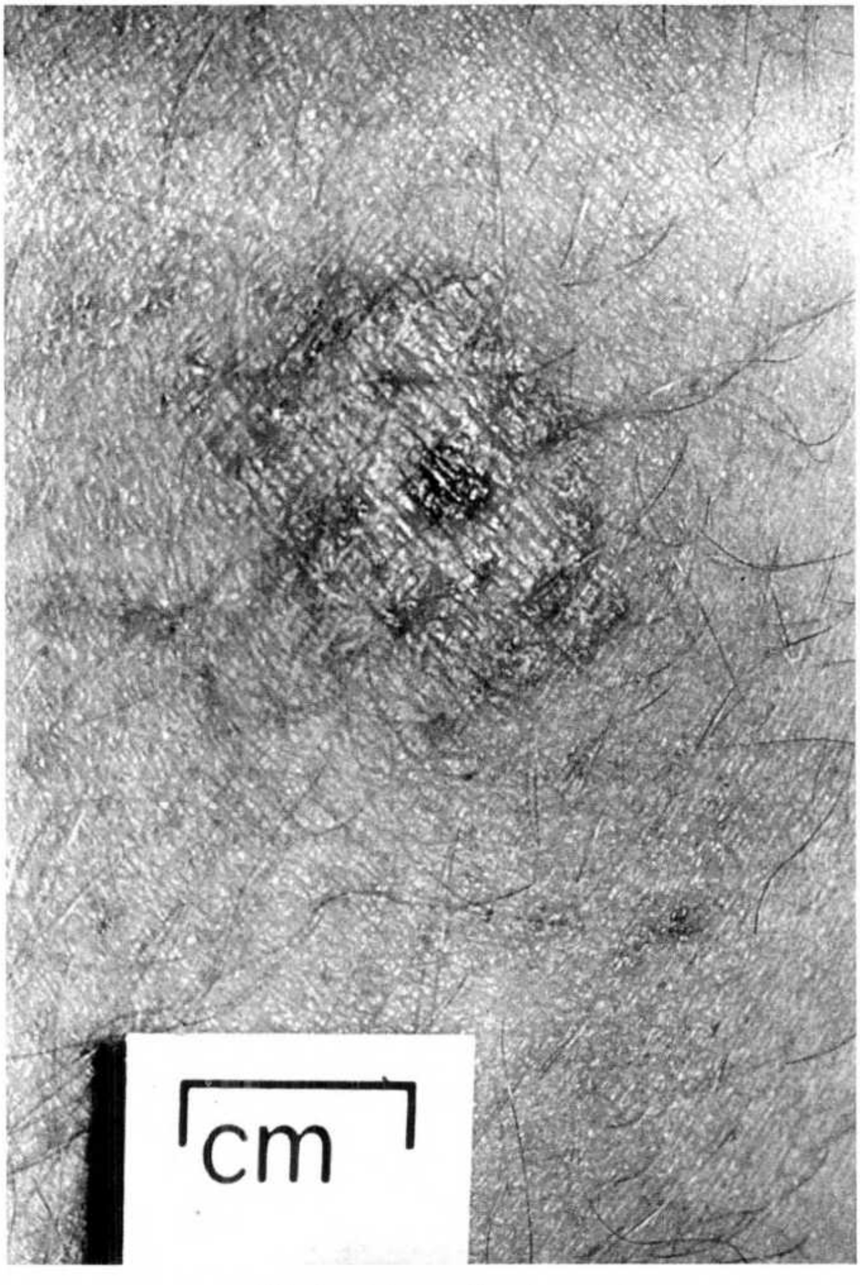

FIGURE 1. Halo dermatitis around a melanocytic naevus.

surrounding subacute eczema with psoriasiform hyperplasia of the epidermis. Collections of normal melanocytes were present in the epidermis, and there was a sub-epidermal lymphocytic infiltrate. The patient had no eczema elsewhere, nor was there a history of atopy. He presented 6 months later with a second naevus that had undergone identical transformation over a period of 2 weeks. The authors suggested that the reaction might be due to a cell mediated immunological

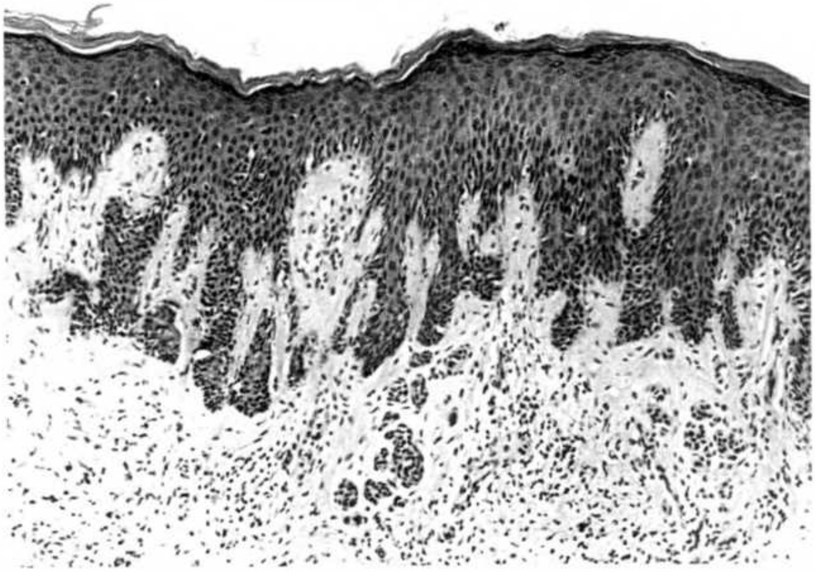

FIGURE 2. Lentigo-like features with naevus cells in the underlying dermis, and an associated inflammatory infiltrate. (H & E; original × 100).

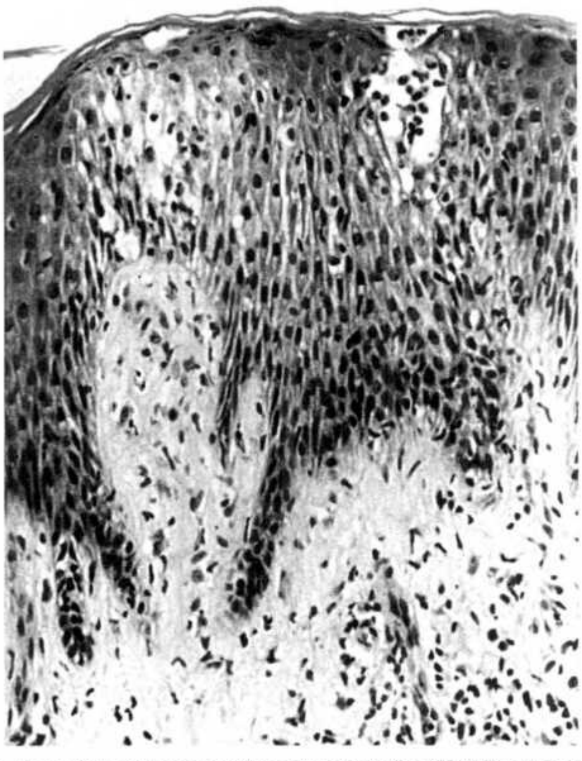

FIGURE 3. Spongiosis with intraepidermal vesiculation (H & E; origianal × 200).

attack, and might be analogous to a halo naevus (leucoderma acquisitum centrifugum, or Sutton's naevus).

Weedon and Farnsworth[3] reported eight cases. They noted the presence of eosinophils in four cases, but found no histological evidence of regression of any of the melanocytic naevi. The persistence of the naevi after the eczematous changes had subsided in the four of their patients who had multiple lesions, indicated that this phenomenom was not related in any way to halo naevi. The authors suggested the change might be that of subacute allergic contact dermatitis.

The most comprehensive review of halo eczema around melanocytic naevi is that of Brenan *et al.*[4] who presented nine patients, seven whom had multiple lesions with areas of eczema surrounding centrally located melanocytic naevi. Of their nine patients, six had no past or family history of atopy. These authors noted that the histology regularly revealed the presence of eosinophils in the dermal infiltrate, and raised the possibility that this may imply a hypersensitivity phenomenon.

Halo-like changes around pigmented melanocytic lesions can take a number of forms. Halo naevus or Sutton's naevus, is an example of a regressing benign naevus with a depigmented zone around the naevus. Halo depigmentation may also occur around a malignant melanoma,[5] and it is important for both clinicians and pathologists to be aware of this possibility. A ring of deeper pigmentation around benign naevi with an intervening narrow non-pigmented zone giving a target-like appearance, has been termed the Cockarde naevus.[6,7] The histological appearance of this lesion shows a central junctional or compound naevus with the peripheral halo usually composed of junctional nests of melanocytes. The non-pigmented zone is usually devoid of naevus cells, and there are no inflammatory changes in the dermis.

Spongiotic (eczematous) changes that are seen histologically may have a number of clinical presentations and have also been described as one of the many features of an epidermal naevus, usually an inflammatory linear verrucous epidermal naevus.[8,9]

Details of all cases known to us are summarized in Table 1.

TABLE 1. Reports of halo dermatitis around melanocytic naevi

Authors	No of cases Total	Multiple lesions	Single lesions	Age range (years)	Sex
Meyerson[1]	2	2	0	22–23	M
Krivanek, Cains and Paver[2]	1	1	0	24	M
Weedon and Farnsworth[4]	8	4	4	19–42	4 M 4 F
Brenan, Kossard and Krivanek[5]	9	7	2	19–49	8 M 1 F
Nicholls and Mason (present report)	1	0	1	21	M
Nicholls (unpublished)	1	1	0	22	F
Total	22	15	7	19–49	16 M 6 F

Halo dermatitis around melanocytic naevi is a benign disorder. It is of importance that of the reported cases at least one patient was thought clinically to have a malignant melanoma. It appears to affect a relatively young age group (average age 30 years) and to have a male predominance, male: female ratio 2·7 : 1. The cause of the condition is unknown. It is a different condition from a halo naevus, as the melanocytic naevus persists despite resolution of the dermatitis either spontaneously or following the use of topical steroids. There appears to be no strong association with atopy. Multiple naevi are involved in about two-thirds of cases and either present together, or separately over a period of time. The lesions appear to undergo spontaneous resolution, in most cases within a few months, without involution of the naevus. The histology is that of a superficial spongiotic dermatitis about a melanocytic naevus. The presence of eosinophils in the dermal infiltrate is a consistent feature.

While this condition is not as common as a halo naevus, it is certainly not rare. Despite this, it is not described in any major dermatological texts to our knowledge. An appropriate eponym for the condition would be Meyerson's naevus, in recognition of the first author to describe halo dermatitis around pigmented melanocytic naevi.

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Correspondence

Complement in chronic cutaneous lupus erythematosus

MADAM, A possible relationship between the different types of chronic cutaneous lupus erythematosus (CLE) and systemic lupus erythematosus (SLE) has been debated for many years.[1,2] This has been supported by the occurrence of various transitional states between the cutaneous and systemic forms of lupus erythematosus and the recent recognition of a distinct lupus erythematosus subset in the transition between CLE and SLE, subacute cutaneous lupus erythematosus (SCLE).[3] The shift from CLE to SLE is reported to occur in 2–20% of cases.[1,2] Therefore, CLE and SLE are suggested to represent forms of the same disease entity. However, the expression of these forms of LE seems to depend on the nature of the host's immune response—in CLE predominantly cell mediated, in SLE predominantly immune complex-mediated.[4] The idea of differences in the pathomechanisms of lesion production in classical CLE and SLE is supported by the restricted effect of thalidomide on cutaneous lesions only in the various types of lupus erythematosus.[5] In contrast to SLE, so far few authors mention complement[4,6] or the incidence of circulating immune complexes[7] in CLE, and to our knowledge no detailed studies have been reported. To elucidate further aspects of the relationship between CLE and SLE, we have studied C_{1q}-binding activity, complement profiles, *in vivo* complement activation and C_4 allotypes in 20 patients suffering from localized CLE on sun exposed areas, mainly the face and scalp–discoid lupus erythematosus (DLE) and five with generalized CLE (GLE) with non-sun exposed areas also involved, and in four patients with the clinical picture of SCLE.

Patients were classified on the basis of clinical findings, light microscopy, immunofluorescence of lesional skin, the criteria of the American Rheumatism Association (ARA) in blood and urine, and subsets of antinuclear antibodies (ANA: ssDNA, dsDNA, RNA, ENA, Sm, SSB). Therapy in all three groups of patients mainly comprized antimalarial agents or thalidomide, and in selected cases etretinate, supported by local corticosteroids. Routine laboratory tests were essentially normal in all three groups of patients, with the exception of a moderate elevation of the erythrocyte sedimentation rate in four of the five patients with GLE, three of the four patients with SCLE and six of the 20 patients with DLE. Positive ANA were found most frequently in SCLE. Lesional immunofluorescence ('lupus band test') with a search for deposits of IgG, IgM, C_{1q}, C_3 and fibrin or fibrinogen gave variable results in all three groups of patients, with no apparent relationship with the other parameters examined.

Using the 'extended' C_{1q}-binding assay[8], pathological C_{1q}-binding activities that could be related to the presence of complexed IgG or IgM were detected in two of the 20 DLE patients, but in none of those with GLE. Two of the four patients with SCLE repeatedly exhibited elevated C_{1q}-binding activity apparently mediated by complexed IgG or IgM, an observation in agreement with the results of Sontheimer *et al.*[9] In the serum of one patient with DLE and one with GLE, elevated levels of non-immunoglobulin mediated C_{1q}-binding were observed during concomitant infections (upper respiratory tract infection and erysipelas, respectively), and in the serum of one patient with SCLE an apparently mixed type of immunoglobulin-mediated and non-immunoglobulin-mediated C_{1q}-binding was evident during an upper respiratory tract infection. Thus, the 'extended' C_{1q}-binding assay is important to exclude false interpretation of pathological C_{1q}-binding activity.

Measurement of total functional complement as well as of alternative pathway haemolytic activity revealed a hypocomplementaemic state in two individuals suffering from long-standing clinically inconspicuous DLE. In these patients the impairment of classical pathway activity was associated with reduced concentrations of C_2 and C_4. One patient with the clinical picture of SCLE repeatedly had significantly lowered levels of C_{1r}, C_{1s}, C_2, C_4, C_4-binding protein, C_6, C_8, and factor H and, as a consequence, had lowered total haemolytic complement activity. Cases of selective diminution of C_{1r}, C_{1s}, C_4-binding protein, C_8 and C_9 in three patients with DLE and one patient with GLE were not detected by the haemolytic screening tests. Therefore, the validity of these tests in evaluating disturbances of the complement system in CLE is restricted to drastically diminished levels of single complement proteins or to the combination of partial diminution of several complement components. Weighted means of total and

TABLE 1. Complement profiles in patients with DLE, GLE and SCLE (values are weighted means)

	DLE	GLE	SCLE	normal range
LPO-C_{1q}	1·4	1·3	8·2	< 5%
CT-C_{1q}	1·3	6·9	2·3	variable
CH_{50}	440	406	351	300–510 U/ml[1]
APH_{50}	42	42	33	22–55 U/ml[2]
C_{1q}	0·11	0·11	0·11	0·07–0·17 g/l
C_{1r}	104	105	95	75–125%[3]
C_{1s}	109	100	94	70–125%
C_2	118	117	85	68–130%
C_{3dg}	5·3	5·2	7·0	< 15%
$C_3(C_{3c})$	1·34	1·17	1·07	0·75–1·40 g/l
C_4	0·25	0·21	0·15	0·14–0·35 g/l
C_5	113	133	109	68–122%
C_6	111	108	109	68–126%
C_7	134	121	108	68–132%
C_8	99	98	99	70–120%
C_9	126	123	127	52–122%
B	120	119	94	60–140%
P	104	103	86	60–135%
C_1-INH				
conc.	0·17	0·21	0·17	0·11–0·26 g/l
funct.	105	107	109	70–135%
C_4-BP	99	140	73	68–137%
H	115	101	94	60–130%
I	113	117	84	60–135%

[1] 1U corresponds to the amount of serum which lyses 50% of 3×10^7 optimally sensitized sheep erythrocytes in 45 min at 37°C in a total volume of 0·5 ml

[2] 1U corresponds to the amount of serum which lyses 50% of 5×10^7 rabbit erythrocytes in 45 min at 37°C in a total volume of 0·5 ml.

[3] From a pool of 150 healthy blood donors

alternative pathway haemolytic activity and of most of the levels of the complement proteins were within the normal range for all three groups of patients (Table 1). The results of Prystowsky and Gilliam[4] showing lowered C_3 and those Schrager and Rothfield[6] showing elevated properdin could not be verified in our CLE patients.

An attempt was made to detect systemic activation of complement in the patient groups investigated. Besides the concentration of C_2, functional C_2 was assessed to ascertain C_2 antigen levels, considered to represent native C_2. Forty sera from 27 randomly selected patients were analysed (18 DLE, five GLE, four SCLE). No accumulation of C_2 breakdown products was detected. Half the normal C_2 antigen concentration and function was found in a patient with SCLE. The highest levels of C_2 concentration and function were observed in a patient with DLE. Activation of the key molecule of the complement system, C_3, was investigated by quantification of the breakdown product C_{3dg}. Elevated levels C_{3dg} were very rare and were not observed consistently. Weighted means of C_{3dg} levels in the different groups of patients are given in Table 1. The formation of the fluid-phase terminal $SC_{5b\text{-}9}$ complement complex (TCC)[10], indicated by the appearance of a neoantigen on C_9, was assessed in 20 plasma samples from 17 randomly selected patients (11 DLE, four GLE, two SCLE), Although markedly elevated C_9 levels could be found in individual plasma samples, in only three of these could a slightly augmented TCC be observed. Two of these samples were from the same patient with SCLE, the third was from a patient with DLE.

Lupus erythematosus has been reported to be associated with complete or partial deficiencies of C_2 and C_4.[11,12] This prompted us to study C_4 allotypes in our patients. Phenotyping of C_4[12] in a limited number of randomly selected patients (seven DLE, two GLE, three SCLE) revealed absence of C_4 gene products in two patients with DLE, one with GLE, and three with SCLE. The most notable patient in our study suffered from SCLE. She repeatedly presented with the highest serum levels of C_{1q}-binding activity, only half the normal concentration of C_2, lowered levels of several complement components other than C_2 and C_4, and slightly raised TCC. Her C_4 genotype was A_3/A_3 B_1/B_{Q0}.

No relationship between the type of lupus erythematosus, the ARA-score, duration or activity of the disease, history of photosensitivity, presence, type or titre of ANA, or the individual complement parameters could be demonstrated. Therapy, particularly with thalidomide, had no apparent influence on the complement profile. We conclude that there is currently no simple laboratory test available which would be of value in clearly subtyping CLE or in distinguishing between CLE and SCLE.

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We thank Mrs L. Meyer-Hänni and Miss I. Bachmann for excellent technical assistance. We are indebted to Drs R. Bütler, G. Hauptmann and A. Krebs for their continued interest, support and critical comments.

Dept of Dermatology,
University of Berne, Berne, Switzerland
* Institute of Immunology and Rheumatology
The National Hospital, Oslo, Norway
** Institute of Haematology and Immunology
University of Strasbourg, France
‡ Central Laboratory of the Swiss Red Cross
Blood Transfusion Service
CH-3000, Berne 22, Switzerland

TH. HUNZIKER
T.E. MOLLNES*
G. MISIANO**
P.J. SPÄTH‡

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Bone X-ray examinations in patients treated with isotretinoin: a prospective study

MADAM, There is currently considerable interest in the mechanism of action of the retinoids and their long-term toxicity. Since acute retinoid toxicity is in many respects reminiscent of hypervitaminosis A, closer examination of long-term side effects on the bones seemed particularly worthwhile. Bone lesions have been reported primarily as a result of several years' treatment with high doses.[1]

We wished to find out whether the therapy given in Europe—moderate doses over relatively short periods—may be considered safe with regard to the skeletal system, and we have carried out a prospective radiological study of the effects of 4 months' treatment with moderate doses of isotretinoin.

Sixteen men with severe acne (acne conglobata or cystic acne) aged 14–42 years (mean, 22 years) were treated with isotretinoin (Roaccutane®), 1 mg /kg daily for 4 months.

Lateral radiographs of the cervical spine were taken before and at the end of treatment and 6 months after the end of treatment.

No radiological changes were found after 4 months of treatment or 6 months after completion of treatment. None of the patients complained of any spontaneous bone, joint or muscle pain, during or after the treatment.

Pittsley and Yoder[2] were the first authors to draw attention to bone changes as a side effect of retinoid treatment. Similar side effects were reported later by other authors.[3–8]

Pennes *et al.*[9], who treated eight patients with isotretinoin, 2·0 mg /kg daily for 9–12 months, found calcification in the soft tissue of the hand in six cases 1 year after ending treatment and on the tarsal bones extending to the heel bone in two patients after 18 months' treatment. Gerber *et al.*[5] found bone changes equivalent to DISH in a higher percentage of patients with basal-cell naevus syndrome and basalioma than in a control group. In their opinion, complications are to be expected when patients receive 2 mg /kg isotretinoin for more than 2 years. Ellis *et al.*[4] described calcifications of smaller size on the os naviculare in one of seven patients 1 year after treatment with isotretinoin (1·8 mg /kg for two 16 week periods), although these did not produce any clinical symptoms. Kilcoyne *et al.*[10], in their investigation of 96 patients treated with isotretinoin, found small, point-like ossifications in the anterior portion of the vertebrae in 10 patients. In the present study, in which patients received 1 mg isotretinoin /kg daily for 4 months, no pathological X-ray changes attributable to the retinoid were found after the end of therapy. On the basis of X-ray pictures taken 6 months after the end of therapy, we were also able to exclude the possibility of bone changes appearing at a later stage. We conclude that retinoid treatment given for this duration and in the stated dosage may be regarded as safe from the point of view of the skeletal system.

Given that retinoid-induced bone changes are not predictable at present, treatment has to be monitored by means of radiological and scintigraphic examinations in addition to careful observation of the skeletal system and joint symptoms. In isotretinoin treatment lasting more than 6 months bone scintigrams and X-ray examinations are of importance. Scintigrams may be of help in choosing the areas to be subjected to radiological examination.

Dermatology Unit and
* Radiology Unit
Bács-Kiskun County Hospital
6000 Kecskemét, Hungary

L. TÖROK
S. BUDAI *
M. KÁSA
L. KÁDÁR

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Etretinate and nodular prurigo

MADAM, The report by Boer and Smeenk (*British Journal of Dermatology*, 1987, **116,** 271) of nodular prurigo developing during etretinate treatment is most interesting. Both their patients had hyperkeratotic eczema of the palms. Both were treated with etretinate and both developed widespread pruritus and nodular prurigo.

In our survey of 47 patients with nodular prurigo, accepted potential causes of pruritus (other than the nodular prurigo itself) were found in a substantial proportion.[1] These were metabolic in 50% (such as anaemia, hepatic dysfunction, uraemia, myxoedema), focal in 37% (insect bites, venous stasis, folliculitis, nummular eczema) and psychosocial in 33%.

It seems that nodular prurigo is induced by scratching highly atopic (or constitutionally eczematous) skin. The cause of the scratching (or pruritus) is immaterial. The nodular prurigo reaction pattern occurs only in highly atopic individuals.

Etretinate may cause occasional generalized pruritus.[2] So, might it be that in Boer and Smeenk's patients the etretinate caused pruritus which led to scratching which, because of the patients' tendency to hyperkeratotic constitutional eczema, resulted in a hyperkeratotic eczematous reaction pattern—nodular prurigo?

Skin and Therapeutics Research Laboratories,
Westminster Hospital,
London SW1, U.K.

C.M.E.ROWLAND PAYNE

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Reply

MADAM, In their article on nodular prurigo (NP), Rowland Payne *et al.* divided patients with NP, according to clinical criteria, into two groups: classical NP (Hyde) and NP in association with eczema. These authors suggested that all forms of NP are closely related to eczema and that the NP reaction pattern only occurs in highly atopic individuals.

In our cases we assumed a relationship between the NP-like lesions and the etretinate treatment, without consideration of the intermediate factors of pruritus, scratching, and atopic skin. We based this on the principle that cutaneous signs of pruritus, if present, are usually associated with excoriations rather than with nodules. The lesions in our patients were described as NP-like eruptions (in contrast to NP lesions) because the lesions were not clinically identical to the typical NP lesions of Hyde. Histopathological investigation cannot provide a definite answer, because chronic eczema, lichen simplex, and NP are regarded as a continuum of histological changes. Although it was not mentioned in our article, our two cases did have atopic features. Indeed, if we were to accept the clinical classification of Rowland Payne *et al.*, who included the NP eczema patients in their study because they had 'an eczematous eruption with prurigo nodules', our patients with NP-like lesions might be considered to belong to the group of NP in association with eczema (NP eczema).

Department of Dermatology,
Deventer Hospitals,
Postbox 5001, 7400 GC Deventer,
The Netherlands.

J.Boer

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Book Reviews

Tradition of excellence. Dermatology at the University of Pennsylvania 1870–1985. H.BEERMAN and G.S.LAZARUS. Obtainable from the Department of Dermatology, University of Pennsylvania. Price $49.00.

There is a tide in the affairs of man, institutions and countries, but there is no ebb and flow in the excellence of dermatology in Philadelphia. The history of dermatology at the University of Pennsylvania, so beautifully presented under the deserving title 'Tradition of Excellence', is a chronicle of success—past and present. Reading it, one could be excused for thinking that one is delving into a Who's Who of dermatology.

The University of Pennsylvania School of Medicine was the first and only Medical School in the 13 American colonies when it was founded in 1765. It was modelled on the University of Edinburgh. There were attempts at introducing dermatology in Philadelphia and in America for the next 100 years, partly from Europe, but mainly from England. Louis Duhring became Philadelphia's first skin specialist in 1870 after training at the University of Pennsylvania and attending clinics in Vienna, Paris and London. Known to us mainly for his work on dermatitis herpetiformis, he meant much more to American dermatology for his building up of the skin department in Philadelphia and for substantial legacies to the department of cutaneous medicine. Weidman, Stokes, Schamberg, Pillsbury, Shelley and Kligman are equally famous in America and on this side of the Atlantic. Herman Beerman (co-author of 'The Tradition of Excellence' with Gerald Lazarus) was not only Chairman of the Graduate Department of Dermatology and a father-figure of the Society of Investigative Dermatology. His faithful and regular visits with Emma Beerman to the British Association of Dermatologists' Meetings have made him a familiar and most popular figure amongst Dermatologists in this country. Even his absence at a meeting recently was commented on and greatly regretted.

Perhaps the most interesting and significant contribution of the Philadelphia School of Dermatology is the widespread progeny of famous sons of this medical school who have moved to new centres and have educated a new generation of dermatologists. Looking down the list of Pillsbury's trainees (page 67) who have headed departments of dermatology, one sees names like Harvey Blank, Shelley, Maibach, John Strauss, Frederick Urbach and others equally eminent and one cannot help being reminded that these have trained yet other famous skin physicians including Steven Katz, Gerry Weinstein, Bill Eaglestein and a host of others. 'The Tradition of Excellence' seems to be self-perpetuating.

I.SARKANY

Skin Disease in Old Age. R.MARKS (1987) London: Martin Dunitz. Pp 276. Price £35.00.

This is an enjoyable, informative text written in a chatty style by a prolific author. It is really a textbook of dermatology and although entitled 'Skin Disease in Old Age' it could almost be a general textbook.

Opening with chapters on structure and function and environmental considerations the remainder of the book is clinical.

My minor criticisms are that references should be at the end of each chapter rather than lumped together at the end of the book. Figure captions should be opposite or under figures rather than grouped at the top or bottom of a page and there are spelling errors such as morphoea repeatedly misspelt on p. 123.

At £35.00 this well-produced, lavishly illustrated book is a good buy and not expensive these days. It will be particularly useful as an introduction to dermatology to medical students and family doctors and to those beginning a career in dermatology.

J.VERBOV

Systemic Lupus Erythematosus. Edited by R.G.LAHITA (1987) New York John Willey. Pp. 974. Price £85.55.

Dubois' Lupus Erythematosus Edited by D.J.WALLACE and E.L.DUBOIS (1987) 3rd edition Philadelphia. Lea and Febiger. Pp 775 including index. Price $98.50.

In his preface, Dr Lahita states: 'If you know systemic lupus erythematosus, then you know medicine'. He has drawn together 52 authors, 50 of whom are American and two Canadian, many of whom were disciples of the late Henry Kunkel, a most distinguished medical scientist and physician, who died in 1983 and to whom the book is dedicated. The main subjects include: aetiology, animal models, autoantibodies and antigens, clinical aspects, drug-induced systemic lupus erythematosus and therapy. There is a lot of information, but unfortunately the references date only up to 1984. In a rapidly-developing field, this is not good enough. In a section on sex and age, one might expect a discussion of the whole subject, but the account is firstly about late onset systemic lupus erythematosus and secondly the influence of sex hormones. The therapeutic section is well-documented, but reads like a pharmacological treatise rather than a practical companion by a knowledgeable physician, and the general care and welfare of the patient is not mentioned, although in the preface it is stated that 'the aim of the book . . . is to avoid harm to the patients by over-aggressive well-intentioned therapy'.

This book should be compared with the publication this year of the third edition of Dubois' Lupus Erythematosus, edited by Daniel J. Wallace and Edmund L. Dubois. This well-established book does not look at all jaded and, despite the 128 pages of references (approximately 5000 references, all with full titles), there is much evidence of the authors' personal experience. In management, they stress the value of rest and are down-to-earth with their statement that 'the management of renal disease is more an art than a science'. They also try to put matters into perspective; for example, they point out that neonatal lupus erythematosus and heart block, although of great interest, is a comparative rarity.

For my money, I would buy Dubois.

N.R.ROWELL

Systemic Lupus Erythematosus. Clinical and Experimental Aspects—Edited by J.S.SMOLEN and C.C.ZIELINSKI (1987) Berlin: Springer. Pp. 354. Price DM. 198.

The editors, both members of the 2nd Department of Medicine of the University of Vienna, have drawn upon the experience of many colleagues from Vienna and across the Atlantic to produce this series of essays on growing points in systemic lupus erythematosus. It is not meant to be a comprehensive textbook, although it deals with historical aspects, animal models, immunology, and genetic and clinical facets. George Tappeiner discusses complement and complement deficiencies, and I enjoyed particularly the chapter on the role of lymphokines. There is no attempt to discuss therapy in general, but there is an excellent account of the place of plasmapheresis, and the authors point out that prolonged remission is only achieved if plasmapheresis is combined with high dose cyclophosphamide. Of course, this treatment should be reserved for serious active cases.

I like this book and would recommend it to all who are interested in a disease which has something for everyone, whether they be clinicians, basic scientists of those who merely speculate on aetiology.

N.R.ROWELL

Radiation Damage to Skin—Fundamental and Practice Aspects (1986). H.JAMMET *et al. British Journal of Radiology* (Supplement No. 19). Pp 155. Price £18.00.

This supplement to the *British Journal of Radiology* reports the proceedings of a workshop in Saclay, France in October 1985 and is divided into seven sections, each including several papers from European and American centres on different forms of radiation damage to the skin. The fascinating chapter dealing with localized irradiation accidents fits in well with the clinical and experimental section which follows. There are several interesting radiobiological dose meters described, the most interesting of which is the growth of dyed plucked hair after varying doses of irradiation. As ever when discussing radiation damage to the skin, the poor pig, whose dermatological profile is apparently most closely related to man, provides much of the experimental data. Although there are some strange suggestions for treating radiation damage and some anomalies of translation, this volume is compelling reading for anyone interested in the combination of skin and radiation.

MARGARET F.SPITTLE

Scanning Electron Microscopy of Normal and Abnormal Human Skin. W.H.WILBORN, B.M.HYDE and L.F.MONTES. 1985, Weinheim, V.C.H., Verlagsgesellschaft, Pp 221. Price DM 145.

Scanning electron microscopy (SEM) provides unique information about surface topography and ultrastructure. This book contains a wide selection of scanning electron micrographs of skin—not only of external appearances of hair and stratum corneum, but also of the cut surfaces of the different layers so that many dermal components, including collagen and elastic tissue, endothelium and fibroblasts are illustrated. The book is more than an atlas of SEM because there is a fair amount of text accompanying drawings and photomicrographs, and scanning and transmission electron micrographs.

The best sections are on preparative techniques and fetal skin. The development and regression of the periderm are beautifully shown. The decision to include sections on such diseases as acne, capillary haemangioma and basal cell carcinoma is hard to understand. The pictures look impressive, but the scientific message is unclear.

The book is reasonably priced and in its way is the most useful general reference on SEM of skin. A lack of coverage of disorders of nail and hair—two structures which lend themselves particularly well to this form of analysis—will weaken its attraction.

R.A.J.EADY

Advanced Dermatological Therapy. W.B.SHELLEY, and E.D.SHELLEY (1985) Philadelphia W.B. Saunders. ISBN 0-7216-1193-1. Pp 607. Price £115.00.

Thirteen years ago while a young consultant, I came across 'Consultations in Dermatology' by Walter Shelley and found it a marvellously refreshing and stimulating look at a number of conditions. Now, 13 years on, Walter Shelley has done it again. This time it is Shelley^2, and from the dedication we may well expect a Shelley dynasty.

There is no doubt whatever that Walter Shelley's writing reaches the parts of the mind that other writers fail to reach or even recognize. The mixture of opinion, stimulating challenging statement, references, quotations and asides is quite unbeatable and keeps one reading much longer than intended.

Now for some facts. The book is correctly termed 'Advanced Dermatological Therapy' and is not the ideal text for the uninitiated. Over 150 disease entities are listed in alphabetical order. Under each, a personal Shelley introduction is followed by selected references which include a brief summary of the contents of these. Problems with the management of acroydinia, cat scratch disease, chancroid, or the Quixote Syndrome? Therapy is within. Looking for witty original quotations with perhaps more than superficial meaning for your next student lecture, letter to the Times or your next after dinner speech? Inspiration is here. The fact that I do not always agree with Shelley is neither here nor there. It is made clear in the title that we are in the realms of opinion and where I disagree I will have been happily stimulated to rethink my own views.

This book should be bought personally by all consultants and senior registrars. It is much better value than the registration fee for a refresher course, and the ideas contained between these pages will keep you thinking for much longer.

RONA M.MACKIE

British Journal of Dermatology (1988) **118**, 140

News and Notices

Consultant Appointments

The following consultant appointments have been notified to the BJD office.

The following were appointed to consultant posts in 1986:

C.M.Lawrence	Newcastle upon Tyne

The following were appointed to consultant posts in 1987:

R.Lever	Glasgow
J.R.Marsden	West Midlands
R.H.Meyrick Thomas	Wessex
B.E.Monk	North Bedfordshire
M.F.Muhlemann	States of Jersey

Australian Dermatopathology Society Ninth Annual Conference

The Ninth Annual Conference of the Australian Dermatopathology Society will be held at the Nusa Dua Beach Hotel, Bali, Indonesia in conjunction with the International Society of Dermatopathology, June 13–15, 1988 preceding the Eighth Regional (Asian-Australian) Conference of Dermatology, June 16–20, 1988. The principal guest speaker will be Professor E.Wilson-Jones of London. For enquiries regarding registration contact Dr P.J.Heenan, 26 Leura Street, Nedlands, Western Australia 6009 (Tel. 09 389 1022) or Dr V.Munro, Department of Histopathology, St Vincent's Hospital, Victoria Street, Darlinghurst, N.S.W. 2010, Australia (Tel. 02 361 2380).

8th CIRD Symposium: Advances in Skin Pharmacology

The 8th CIRD Symposium Advances in Skin Pharmacology will be held in Cannes, France, September 1–3, 1988. The topic will be Pharmacology of Retinoids in the Skin. The meeting topics will cover both basic and applied aspects of retinoids, and will begin with an afternoon workshop dealing with retinoid modulation of epidermal differentiation. The remaining 2 days will consist of three plenary sessions dealing with treatment of skin diseases, prevention and treatment of skin damage, new active chemical entities and potential new therapies.

For further information contact: Dr B.Shroot, Centre International de Recherches Dermatologiques, Sophia Antipolis, F-06565 Valbonne, France. Telephone-93.95.70.70, Telex-461 030.

Deadline for submission of abstracts for papers or poster presentations 31 March 1988.

Second International Workshop on Langerhans Cells

The Second International Workshop on Langerhans cells will be held at the International Agency on Cancer Research (Center International de Recherches sur le Cancer), Lyon, France, April 21–22, 1988. The topics presented will include Langerhans cell origin and differentiation, surface antigens and markers, *in vivo* and *in vitro* functions, Langerhans cells and grafts, Langerhans cells and contact dermatitis, Langerhans cells and AIDS, Langerhans cells and other diseases. The program will include formal presentations and posters.

For further information please contact the organizers: J.Thivolet and D.Schmitt, Clinique Dermatologique (Pav.R), Hopital Ed. Herriot, 69473 Lyon, Cedex 03 France.

British Journal of Dermatology (1988) 118, 141–155.

Review

The pathomechanisms of psoriasis; the skin immune system and cyclosporin

J.D.BOS

Department of Dermatology, University of Amsterdam, Academisch Medisch Centrum, Amsterdam, The Netherlands.

Accepted for publication 24 August 1987

Psoriasis is a common and chronic disease with a complex heredity and an as yet unknown aetiology. One or more primary and genetically determined defects must be present, but have not been identified as yet. Skin symptoms are almost always present while a minority of patients have one of five forms of arthritis.[1]

Since the publication of the excellent review on the pathogenesis of psoriasis by Krueger,[2] a large number of publications has extended our knowledge of the secondary and tertiary pathomechanisms involved. The present review presents the investigational approaches under three headings: cellular, intercellular and intracellular. The possible factors involved are listed in Table 1. As far as intracellular pathomechanisms are concerned, emphasis is placed on the plasma membrane signal transducing systems. The similarity between the cellular subtypes involved in psoriasis and the constituents of the skin immune system[3] is indicated. The six possible modes of action of the recently introduced antipsoriatic drug cyclosporin, a drug generally in use as an immunosuppressant, are then described.

It must be stressed that, at present, psoriasis has still not been proved definitively to be a primary immunological disease. Circumstantial evidence supporting the immunological hypothesis is increasing however, as can be seen from some of the recent developments in psoriasis research described here.

PATHOGENESIS OF PSORIASIS AT THE INTRACELLULAR LEVEL

Studies directed at the detection of a biochemical defect in psoriatic skin have revealed a number of abnormalities related to transmembranous signal transducing systems (Table 2; Figs 1 and 2). These include reduced cAMP cascade activity,[4] high lesional cyclic guanosine monophosphate (cGMP) levels,[5] increased calmodulin levels,[6,7] increased phospholipase C (PLC) activity,[8] increased tyrosine kinase activity,[9] and increased epidermal growth factor (EGF) binding in the upper layers of involved epidermis.[10]

Part of this review was presented at the First International Workshop on Psoriasis and Cyclosporin, Amsterdam, June 1987.

Correspondence: Dr J.D.Bos, Department of Dermatology, University of Amsterdam, Academisch Medisch Centrum, AO–223, Meibergdreef 9, 1105 AZ Amsterdam, The Netherlands.

TABLE 1. Possible components of the pathomechanism of psoriasis.

Cellular	Intercellular	Intracellular
fibroblasts	epidermal growth factor (EGF)	phospholipase A_2
keratinocytes	leukotrienes: 12-HETE, LTB_4	guanylate cyclase–cGMP
endothelial cells	prostaglandins: PGE_2, $PGF_{2\alpha}$	adenylate cyclase–cAMP
granulocytes	platelet activating factor PAF	phospholipase C–DG/IP3
monocytes	interleukins: IL-1	tyrosine kinase
dendritic cells	interferons: IFN-α, IFN-γ	calmodulin
T lymphocytes	complement C_{5a} des arg	ornithine decarboxylase

PLC activity in psoriatic lesions is markedly increased compared with uninvolved and normal skin.[8] Activated PLC forms diacylglycerol (DG) and inositol 1,4,5-triphosphate (IP3) from the plasma membrane, and these products act as secondary messengers in cell activation and replication.[11,12] Activated PLC may induce guanylate cyclase which by the formation of cGMP additionally initiates cellular responses. A wide variety of external stimuli including antigens, some growth factors, neurotransmitters, certain hormones and platelet activating factor can induce this inositol phospholipid secondary messenger system in various tissues.

It is known that adenylate cyclase, guanylate cyclase, phospholipase C and tyrosine kinase all act in the regulation of cell proliferation and differentiation by their effects on intracellular protein phosphorylation. Thus the transmembranous signal transducing systems adenylate cyclase–cAMP, guanylate cyclase–cGMP, phospholipase C–DG/IP3, and EGF–tyrosine kinase are all altered in involved psoriatic skin. This is associated with protein activation (ornithine decarboxylase and phospholipase A_2), an increase in cell proliferation, and abnormal keratinocyte differentiation. A report describing increased ornithine decarboxylase (ODC) activity[13] could not be confirmed by Bouclier *et al.*[14] although tape-stripping was found to give a significant increase in both involved and uninvolved psoriatic skin compared with normal skin. ODC induced polyamines spermidine and spermine were found to be elevated.[14]

It must be stressed that many of these observations were made in keratom sections of lesions; the exact cellular sources of the changes in signal pathways remain to be established. Studies describing biochemical abnormalities in psoriatic epidermis have never completely excluded the possibility that at least some of the findings could be explained on the basis of the presence of immunocytes in the epidermal cell samples studied. The recent finding of increased cAMP-phosphodiesterase as well as PLC activity in mononuclear leukocytes from peripheral blood of psoriasis patients[15] seems to support this suggestion. In this context, we have found the number

TABLE 2. Transmembrane signal transducing systems possibly involved in the pathogenesis of psoriasis.

Typical agonist	Plasma membrane amplifier	Secondary messenger
adrenalin	adenylate cyclase	cyclic AMP
?	guanylate cyclase	cyclic GMP
antigen	phospholipase C	diacylglycerol inositol-triphosphate
epidermal growth factor	tyrosine kinase	

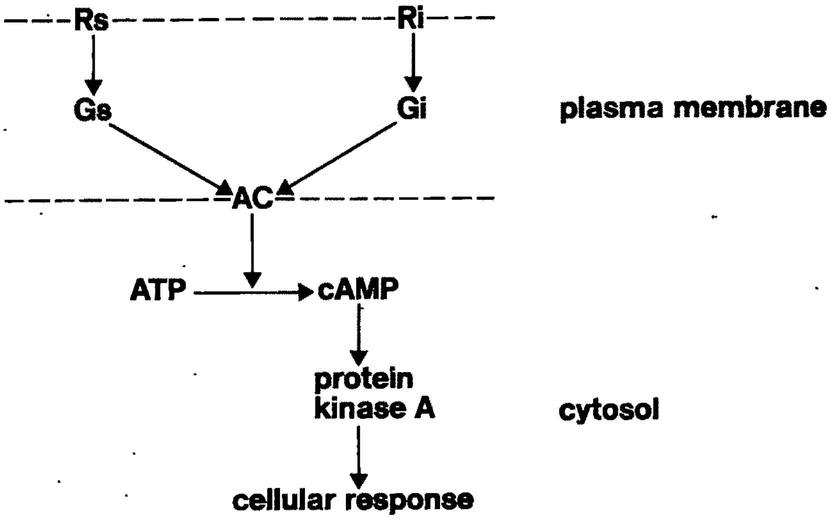

FIGURE 1. The adenylate cyclase–cAMP pathway. Rs = stimulatory receptor, Ri = inhibitory receptor, Gs = stimulatory G protein, Gi = inhibitory G protein, AC = adenylate cyclase.

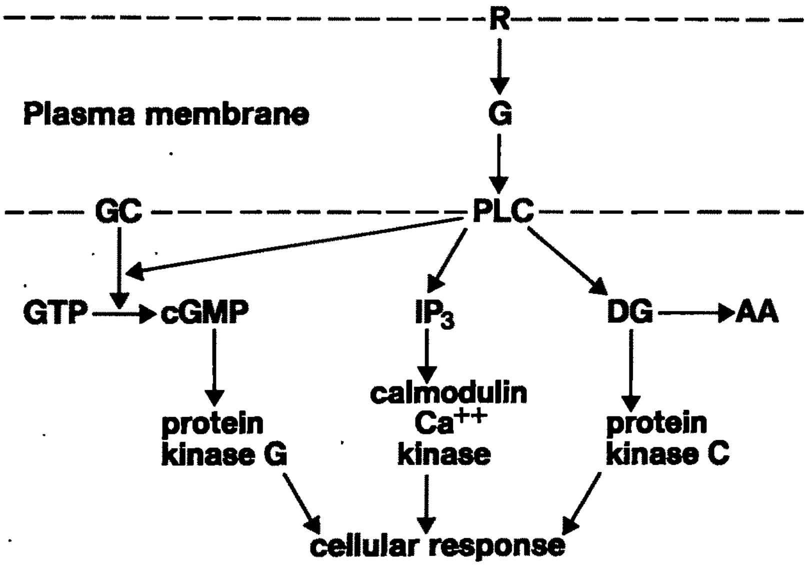

FIGURE 2. The phosphoinositol pathway. PCL = phospholipase C, AA = arachidonic acid, R = receptor, G = G protein, GC = guanylate cyclase, IP3 = inositol-triphosphate, DG = diacylglycerol.

of intra-epidermal T lymphocytes to be higher in psoriatic epidermis (up to 90,000/cm^2) than in the epidermis of atopic dermatitis, pityriasis rosea, nummular dermatitis and lichen planus.

PATHOGENESIS OF PSORIASIS AT THE INTERCELLULAR MEDIATOR LEVEL

Krueger & Jederberg[16] demonstrated that supernatants of mononuclear cells had both inhibitory and enhancing effects on HeLa cell cultures. Those obtained from psoriasis patients, however, had less inhibitory and more enhancing effects, suggesting that epidermal hyperplasia in psoriasis may result from an imblance in mediator release. Korszun, Wilton and Johnson[17] injected lymphokines obtained from guinea pig stimulated lymphocyte supernatants intracutaneously and observed increased mitotic activity in the epidermis. A partially purified lymphokine also induced parakeratosis. The mononuclear cell derived lymphokines that thus seem able to reproduce some of the characteristics of psoriatic epidermis have been called 'epidermal proliferating factors' (EPF).[18] The mediators described below may contain the molecules responsible for cell activation effects present in psoriasis. In addition, a number of other substances such as hormonal peptides, epidermal growth factor (EGF) and neurotransmitters (substance P[19]) may be involved although definite proof is not available.

Eicosanoids: prostaglandins and leukotrienes

Arachidonic acid (AA), the precursor of a family of inflammatory and mitogenic mediators collectively known as the eicosanoids, is increased in psoriatic skin. Of these eicosanoids, the lipoxygenase products 12-hydroxy-eicosatetraenoic acid (12-HETE) and leukotriene B_4 (LTB_4) and to a lesser extent, the cyclo-oxygenase products prostaglandins PGE_2 and PGF_{2a} have been found to be elevated in psoriatic lesions[20-24] (Fig. 3). It has been suggested that psoriatic skin might contain an endogenous inhibitor of cyclo-oxygenase, resulting in the diversion of arachidonic acid to the lipoxygenase pathways. The slight increase in PGE_2 and PGF_{2a} may be partly responsible for vasodilation and perhaps also for keratinocyte hyperproliferation.[25]

LTB_4 is known as one of the most potent chemoattractants for polymorphonuclear leukocytes (PMNL). Normal, involved and uninvolved skin all show intra-epidermal microabcesses consisting of PMNLs after epicutaneous application of as little as 50 ng LTB_4. Psoriasis,

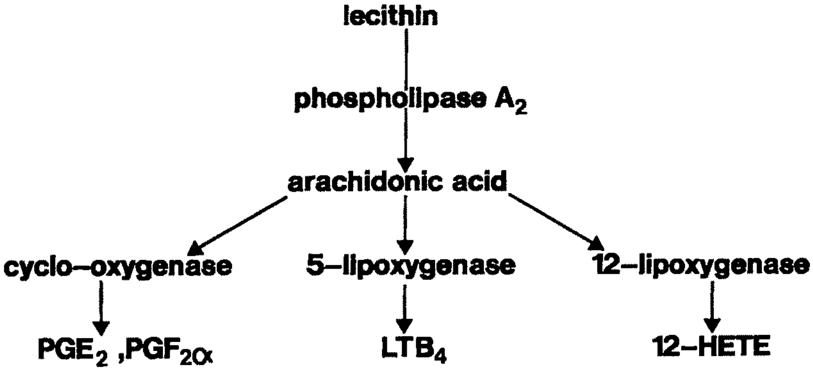

FIGURE 3. Simplified arachidonic acid pathway.

however, does not develop in uninvolved skin following the application of LTB_4, indicating that leukotriene formation is not likely to be a primary pathogenetic event.[26] In addition, LTB_4 levels have also been found to be increased in allergic contact dermatitis[27] and in atopic dermatitis,[28] both diseases where PMNL infiltration is not apparent. Another effect of increased LTB_4 might be induction of keratinocyte proliferation. Although Wong *et al.*[26] did not confirm this *in vivo*, LTB_4 has been shown to stimulate keratinocyte growth *in vitro*.[29]

It was believed initially that increased AA levels and subsequent production of eicosanoids was primarily the result of increased phospholipase A_2 (PLA_2) activity in the psoriatic lesion. It was found, however, that PLA_2 is also elevated in uninvolved psoriatic skin. Recent evidence points to an alternative or additional source of increased AA, diacylglycerol (DG). As described above, this secondary messenger is produced by phospholipase C from the plasma membrane after receptor-ligand interaction. DG may subsequently be metabolized into AA (Fig. 2).

Platelet activating factor

In addition to the chemoattractants for granulocytes described above, platelet activating factor (PAF) has also been found in psoriatic lesions.[30] PAF, 12-HETE and LTB_4 are cell membrane-derived products of increased phospholipase A_2 (PLA_2) activity, which has been demonstrated in uninvolved and lesional psoriatic epidermis. PAF may activate phospholipase C with subsequent activation of a particular cell function.

Interleukins

Interleukin-1 (IL-1) is now known to have at least two different subtypes, IL-1-alpha and IL-1-beta.[31] The mediator was first known as a thymocyte proliferation factor. It became clear that most nucleated cells can produce IL-1 activity and that IL-1 stimulates the growth and differentiation of a wide array of cell types, including lymphocytes and keratinocytes. There is preliminary evidence that epidermal thymocyte activating factor (ETAF) may be a third class of IL-1.[32]

Recent studies have demonstrated interleukin-1 (IL-1) in psoriatic plaques and scales.[33,34] Although the exact cellular source of this IL-1 is as yet unclear, Langerhans cells, granulocytes and keratinocytes may be responsible. The observed IL-1 may be produced upon stimulation by T cell-derived gamma interferon, and may be partly responsible for additional granulocyte immigration and increased inflammatory signs including erythema and oedema.[35] In addition IL-1 may stimulate keratinocyte growth and induce lymphocyte activation in the epidermis. IL-1 may also activate other cells in the psoriatic lesion such as fibroblasts and endothelial cells.

There have been no studies of the presence or activity of other interleukins (IL-2, IL-3 and IL-4), but it seems likely that II-2 levels, at least, will be found to be increased due to the presence of activated T cells in the lesional skin.

Interferons

Bjerke *et al.*[36,37] have demonstrated gamma-interferon as well as minute quantities of alpha-interferon in suction blister fluid from psoriatic lesions. This may be the product of activated T cells (gamma-interferon) and monocytes/macrophages (alpha-interferon) in involved skin. Gamma-interferon may induce and increase MHC II (HLA-D) expression in a variety of cells, including keratinocytes, Langerhans cells and endothelial cells. It may also have trophic effects on these endothelial cells.[38] As described above, it may stimulate IL-1 production. Exogenous alpha-interferon has been found to be associated with induction of psoriasis.[39]

Complement factors

It has been suggested that C_{5a} is formed as a result of complement activation by anti-stratum corneum antibody–stratum corneum antigen complex formation.[40] It has been shown recently that it is C_{5a} des arg that is present in psoriatic scales.[41] The specificity of the presence of C_{5a} and other complement products has been questioned however. Kimura and Nishikawa[42] provided evidence that the presence of immunoglobulin and complement intercellularly in the parakeratotic horny layer of psoriatic skin is the result of plasma protein coagulation and not an antigen-antibody reaction. Then Johannesson, Hammar and Sunqvist[43] demonstrated that keratinocyte hyperproliferation precedes the deposition of immunoglobulins and complement by one to several days. Immunoglobulin and complement deposition thus may be secondary events during psoriatic lesion formation. Nevertheless, C_{5a} is a neutrophil activating peptide and may be partly responsible for the possibly tertiary phenomenon of PMNL migration into psoriatic epidermis.

PATHOGENESIS OF PSORIASIS AT THE CELLULAR LEVEL

The abnormalities of histogenetically different cell populations in psoriatic skin are difficult to summarize. In general, cells of the skin immune system and fibroblasts all seem to be in an activated state. A schematic representation of the cellular interactions involved in the pathogenesis of psoriasis is shown in Figure 4.

Fibroblasts

The interrelations within the skin, especially between the dermal and the epidermal compartments, have been judged relevant in the search for the pathomechanisms of psoriasis. Fibroblasts, quantitatively the major constituent of the dermis have, therefore, been studied in psoriasis. There is evidence of increased urinary excretion of hydroxyproline and uronic acids,[44] a greater content of glycosaminoglycans,[45] and less cross-linked collagen.[46] Fibroblasts explanted from both uninvolved and lesional psoriatic skin showed persistent hyperprolife-

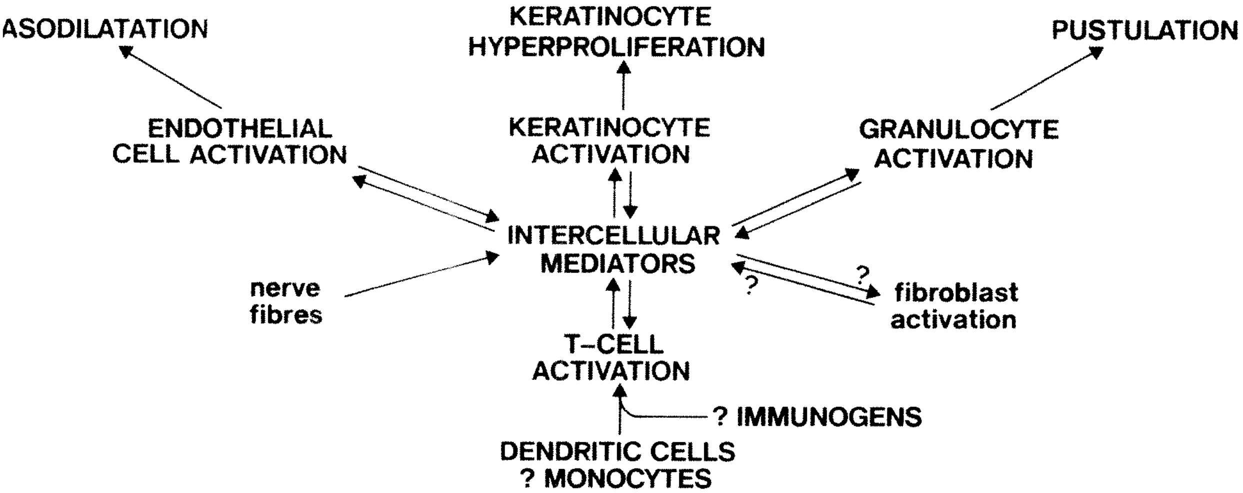

FIGURE 4. Possible interactions between the cellular constituents thought to be involved in the pathogenesis of psoriasis. The actual initiator of these events has not been elucidated.

ration and increased glycosaminoglycan and collagen production *in vitro.*[47,48] Fibrosis, however, is not apparent in psoriatic skin.

The search for the primary site of the defect in psoriasis has led to studies in which lesional and uninvolved psoriatic skin and normal human skin were transplanted onto nude (immune defective) mice and studied for persistence of psoriatic characteristics such as epidermal transit time. Briggaman and Wheeler[49] and Fraki, Briggaman and Lazarus[50] reported combined graft studies in nude mice, where only in the combination of psoriatic epidermis and psoriatic dermis did the characteristics of psoriasis persist. Using another model, Baden, Kubilus and MacDonald[51] found no increase in keratinocyte growth from skin explants cultured on a feeder layer of normal or psoriatic dermal fibroblasts.

Saiag *et al.*[52] describe a series of elegant studies in which full thickness grafts of normal, uninvolved psoriatic and lesional psoriatic skin were explanted in a dermal skin equivalent model *in vitro*. Hyperproliferation of normal explanted epidermis could be induced by fibroblasts from uninvolved as well as from involved psoriatic skin. On the other hand, normal human skin-derived fibroblasts were unable to suppress the hyperproliferative activity of explanted involved psoriatic skin.

These results are controversial and may be artefactual as they are based on data obtained *in vitro*. Both explant and transplant experiments have the additional disadvantage that they disregard the complexity of skin and reduce it to essential cellular components such as keratinocytes and fibroblasts. In all these experiments both epidermal and dermal constituents of the skin immune system[3] are co-transplanted, and some of them may have had profound effects on the results obtained.

Keratinocytes

Many of the approaches described so far have focussed on one of the main characteristics of the cutaneous disease, epidermal hyperproliferation and dyskeratinization. The 12-fold increase in basal and suprabasal keratinocytes in cell cycling[53] is clinically apparent as thickening and scaling. In uninvolved skin, an increase of up to 2·5-fold in the pool of proliferating keratinocytes has also been demonstrated.[54] Therefore, an intrinsic abnormality in psoriatic keratinocytes leading to increased proliferation has been widely sought.

Glycosylation of psoriatic keratinocyte cell plasma membrane proteins may be altered. Studies by Gommans *et al.*[55] have shown that there are either structural changes in the oligosaccharide moieties (especially fucose) on particular glycoproteins, or changes in the relative amounts of different glycoproteins. A primary defect in glycosylation of membrane proteins in keratinocytes thus may be possible. This may result in abnormal keratinocyte plasma membrane receptors with an increased affinity for immune mediators, resulting in keratinocyte stimulation and proliferation.

One important drawback of an exclusively keratinocyte based theory is that it does not account for psoriatic arthritis. Nevertheless, any valid hypothesis must explain the major aspect of the disease, epidermal hyperproliferation.

Endothelial cells

Endothelial cells are abnormal in involved psoriatic skin, especially the most superficial venous capillaries in the upper papillary layer. These vessels have an increased permeability,[56] are dilated and tortuous,[55,56] and show enhanced endothelial cell proliferation.[58] In psoriasis, both keratinocytes and peripheral blood mononuclear cells[60] have been demonstrated to contain angiogenic factors. These might well be interleukin-1. Recent evidence indicates that gamma-interferon may cause trophic stimulation of microvascular endothelial cells, as already discussed.

Granulocytes and monocytes

One of the characteristic histopathological features of involved psoriatic skin is the subcorneal accumulation of polymorphonuclear leukocytes (PMNL) leading to Munro's micro-abcesses, and in severe cases to clinically apparent sterile pustulation. Tagami *et al.*[30] recently reviewed the literature on the presence of potent PMNL chemo-attractants in the horny layer of involved psoriatic skin. Monocytes are part of the subepidermal infiltrate[61] but their exact role is as yet unclear.

Dendritic accessory cells

The dermis in psoriasis is characterized by an accumulation of dendritic cells with the capacity to function as accessory or antigen-presenting cells in a cell mediated immune response.[61–64] The presence of antigen presenting cells of dendritic morphology and bearing a Langerhans cell and interdigitating cell immunophenotype was found to be characteristic of although not entirely specific for psoriasis.[64] Approximately equal numbers of dendritic cells (as a percentage of infiltrating mononuclear cells) were observed in psoriasis and in atopic dermatitis. We have found that the absolute number of cells infiltrating the papillary dermis was much higher in psoriasis than in atopic dermatitis. We conclude that in psoriasis, the absolute number of dendritic antigen presenting cells is higher than in atopic dermatitis and the other dematoses in which these cells were counted,[64] pithyriasis rosea, nummular dermatitis and lichen planus.

Dendritic cell abnormalities in the epidermis of involved psoriatic skin have also been described. A decrease and abnormal clustering of Langerhans cells has been demonstrated by ATPase staining[65] and the monoclonal antibodies anti-Ia[2] c.q. anti-HLA-DR[61,66] and anti-T6.[61,67]

T lymphocytes

The subepidermal infiltrating cells in psoriatic skin lesions have been identified by the use of monoclonal antibodies to define immunocompetent cell subsets. T cells were found to be the major infiltrating cell type.[61,68–70] *In situ* ratios of helper/inducer to cytotoxic/suppressor T-cells vary with the clinical type of psoriasis studied. Chronicity seems to be characterized by a relative decrease in the suppressor/cytotoxic T cell subset.[63] Other studies have indicated that these T cells are activated, since a substantial proportion of them express HLA-DR.[61,66,71] In addition, exocytic infiltration of symptomatic epidermis by T lymphocytes of both major subsets has been described.[61,72]

TABLE 3. Possible immunogens in psoriasis.

- auto-antigens
  - cross reactive epitopes
    - streptococcal antigens
    - mycobacterial antigens
  - stratum corneum antigen
  - non-histone proteins
- allo-antigens
  - retrovirus protein P27

Further subdivision of the CD4+ inducer T cell subset into CD4+, 4B4+ helper inducer and CD4+, 2H4+ suppressor inducer subpopulations has shown that in normal human skin[73] as well as in inflamed skin,[74] helper inducer T cells form the majority. This was also found in psoriasis. Applying what is known about the helper and suppressor inducer T cell subpopulation *in vitro*, it was concluded that the predominance of the helper inducer subpopulation suggests soluble antigen responses by these T cells. This is especially so in psoriasis, in view of the relatively large number of accessory cells present subepidermally, as described above.

THE SKIN IMMUNE SYSTEM IN THE PATHOGENESIS OF PSORIASIS

In the immunological hypothesis, hyperproliferation of lesional psoriatic skin is thought to be induced by the effects of an immune response to as yet unidentified epidermal, dermal or circulating immunogens. Cross-reactivity of inducing allogeneic epitopes and psoriatic skin epitopes may occur. Suggested possible immunogens include non-histone proteins,[76,76], stratum corneum antigens,[40] streptococcal antigens,[77] mycobacterial antigens, and a retroviral protein[78] (Table 3).

A possible role for the immune system in the pathogenesis of psoriasis was indicated by Epstein.[79] Major hypotheses on an immunological pathogenesis of psoriasis were formulated by Cormane *et al.*[80] who suggested a T suppressor cell defect and by Beutner *et al.*[40] who suggested a hidden stratum corneum antigen. Cormane's hypothesis suggests a defect at the T cell level, whereas Beutner's hypothesis focuses on complement activation and neutrophilic infiltration. We have suggested a possible role for dendritic cells in the disturbance of immune homeostasis.[61] Valdimarsson *et al.*[18] gave another variant of the immunological hypothesis by focussing on the intra-epidermal interaction of keratinocytes, T cells and dendritic cells.

One of the major drawbacks of the immunological hypothesis is that the supposed interaction of as yet unidentified immunogens, dendritic cells and T lymphocytes suggests the occurrence of a delayed type of cell-mediated response in the lesional skin, while in psoriasis, there are no histological or clinical signs of the classical type IV response, allergic contact dermatitis. However, there are other examples of dermatological diseases where immunohistology has shown the presence of inducer T cells in association with dendritic cells, pityriasis rosea, atopic dermatitis and mycosis fungoides, which also lack the characteristics of allergic contact dermatitis.

With the exception of fibroblasts, all cells, including keratinocytes, thought to be involved in the pathogenesis of psoriasis, form part of the recently defined 'skin immune system'.[3] It seems justified to conclude that cutaneous psoriasis may in fact be an immunocyte disease. A similar imbalance may occur, in some patients, in the synovia which, when inflamed, contain similar infiltrates consisting of dendritic cells and T lymphocytes. This lack of homeostasis through as yet undefined imbalances in immunoregulatory mediators seems to be associated with activation and proliferation of most cell types studied (Fig. 4). Biochemical evidence for such a state of activation as described above is in accordance with this view.

CYCLOSPORIN AND THE PATHOGENESIS OF PSORIASIS

Cyclosporin (CyA) is a cyclic endecapeptide of fungal origin used as an immunosuppressant. Its antilymphocytic action was first described by Borel *et al.*[81] CyA is an effective and relatively safe drug for the induction of remission in severe psoriasis vulgaris.[82–91] It was also shown to be effective in a case of acute generalized pustular psoriasis.[92]

CyA is not a cytotoxic or cytostatic agent and it does not appear to inhibit growth of keratinocytes[93] although it may inhibit malignant squamous cell lines.[94] CyA does not seem to affect human neutrophil function.[95,96] To my knowledge, no reports of CyA affecting fibroblast function have been published. A preferential effect on lymphocytes and dendritic cells thus seems plausible, although an inhibitory effect on other abnormal psoriatic cell types cannot be excluded. There appear to be six possible ways in which CyA could exert its effect as an anti-psoriatic drug.

Ca^{++} signal modulation. The observed increase of intracellular calmodulin[6,7] in involved skin may be relevant, as calmodulin forms part of the phosphoinositol pathway. As CyA is a calmodulin antagonist *in vitro*,[97] it seems possible that at least part of the effectiveness of CyA in inducing remission of psoriasis may be due to its action as a calmodulin antagonist *in vivo*. However, other calmodulin antagonists, including phenothiazines (trifluoperazine and chlorpromazine) and mepacrine[98] have not been demonstrated to be effective in psoriasis. In fact, propranolol, another calmodulin antagonist, can induce or exacerbate psoriasis. A recent study, however, indicated that dithranol (anthralin), used for local treatment of psoriasis, is a calmodulin antagonist.[99]

Inhibition of membrane phospholipid metabolism. CyA has been demonstrated to inhibit phospholipase A_2[100] which has been shown to be increased in involved psoriatic epidermis, as described above. In this way, CyA may decrease the formation of AA metabolites that are at least partially responsible for the acute component of inflammation in psoriatic skin.

Interference with cell surface receptors. CyA seems to act as a prolactin antagonist at the T cell level.[101] In this way, it decreases ornithine decarboxylase activity and thereby inhibits polyamine synthesis. Thus cell proliferation is inhibited, although it is not clear which cells in psoriasis have increased inducible ornithine decarboxylase activity, as described above.

Inhibition of lymphokine production. It is now generally accepted that CyA affects activated T cells.[102] Although the exact manner in which CyA exerts its anti-T cell activity remains to be elucidated, there is no doubt that it interferes with continuation of lymphokine release by blocking transcription of the genes coding for gamma-interferon and for IL-2.[103] It thereby inhibits further activation and recruitment of effector cells.

Inhibition of MHC II expression. The inhibition of gamma-interferon production by activated T cells, causes a decrease in MHC II (HLA-D) expresion.[104] In this way, interaction of T cells with MHC II positive accesory and antigen presenting cells will diminish, thus further decreasing immunological reactivity and lymphokine release.

Accessory cell inhibition. Evidence provided by several groups[105–107] indicates that CyA alters the antigen presenting capacity of rodent antigen presenting cells, especially that of dendritic cells. Preliminary studies (M. Teunissen; personal communication) provide evidence that accessory cell-function of isolated human Langerhans cells is also inhibited by preincubation with CyA.

CONCLUSIONS

In psoriasis there is an increase in and activation of many cell types in the skin lesions, especially immunocytes. This is reflected in the intracellular biochemical abnormalities that have been observed in the transmembranous signal transducing systems, adenylate cyclase–cAMP, guanylate cyclase–cGMP, phospholipase C–DG/IP3, and EGF–tyrosine kinase. Such a state of activation is associated with the generation of arachidonic acid metabolites (eicosanoids) which tend to increase or perhaps even initiate inflammation and possibly keratinocyte hyperproliferation. The skin immune system shows a number of abnormalities, of which the increase in subepidermal dendritic cells and the high number of T cells infiltrating the epidermis appear disease specific. A wide variety of mediators (interleukins and interferons) that also play a role in keratinocyte hyperproliferation and inflammation, is produced by the cells of the skin immune system. The possible immunogens responsible for these events, however, have not been identified. The lack of inhibition of certain mediator effects is also not understood. Cyclosporin seems to inhibit several activation pathways. The particular success of the drug in psoriasis may be due to a synergistic interaction with some of the pathomechanisms described here.

It should be emphasized that all pathomechanism studies described here are dealing with secondary and tertiary events. The primary defect and thus the aetiology of the disease is still an enigma. Whether there is an intrinsic abnormality in the signal transducing systems of the keratinocytes making them sensitive to immune mediators or, alternatively, whether there is an intrinsic abnormality in the immunocytes, making them especially prone to immunocyte–keratinocyte interaction and stimulation, is still unclear.

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Cantharide acantholysis: endogenous protease activation leading to desmosomal plaque dissolution

B.BERTAUX, C.PROST, M.HESLAN AND L.DUBERTRET

Laboratory of Dermatology, Hôpital Henri Mondor, Creteil, France

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SUMMARY

Using a method which allowed us to study the morphological consequences of the expression and the inhibition of proteases in living tissues, we demonstrated that the primary detectable cellular event in cantharide acantholysis is the dissolution of the dense plaque, leading to the detachment of tonofilaments from desmosomes. This process is inhibited by neutral serine protease inhibitors.

This suggests that the desmosome–tonofilament complex, more precisely the desmosomal dense plaque, is the primary target of activated proteases during cantharide acantholysis, and can be disrupted by a specific epidermal protease–anti protease system. Cantharide acantholysis may be a useful model for studying desmosomal turnover.

The biochemical events leading to epidermal acantholysis are poorly understood[1] (for a general review of acantholysis see Fritsch and Elias[2]). In establishing the role of epidermal proteases in pemphigus vulgaris, several authors have renewed interest in the pathogenesis of acantholytic disorders.[3–7]

Topical cantharide application produces an intraepidermal acantholytic blister,[8] which may be the result of enzymatic processes.[9] Incubation of 0·7 mm thick slices of skin with and without various protease inhibitors prior to fixation has been shown to be an efficient technique for identifying the morphological consequences of endogenous protease activation in human skin.[10]

In the present study we have used this approach to investigate the early morphological events leading to cantharide acantholysis and the involvement of specific activated endogenous proteases in the alteration of the desmosome–tonofilament complex.

Correspondence: Professor L.Dubertret, Laboratory of Dermatology U312, Department of Dermatology, Hôpital Henri Mondor, 94010 Creteil, France.

METHODS

Normal human skin was obtained from four patients undergoing plastic surgery. Skin samples, 4 cm^2 were pinned onto wet filter paper on cork plates and put into Petri dishes containing Hank's medium to maintain a humidified atmosphere. An alcoholic solution of cantharide (0·1 g/ml) was freshly prepared from cantharide extract and filtered.[11] The cantharide solution was spread over the stratum corneum of the skin samples which were then incubated at 37°C (C samples). Some samples were kept at 37°C without cantharide application as controls (T samples). Punch biopsy specimens 6 mm diameter were taken after 1, 2 and 3 h (C1, C2, C3 and T1, T2, T3). Each biopsy was immediately cut into 0·7 mm slices using a hand microtome to test for protease inhibitors.[10] These slices were treated as follows: one slice was fixed immediately (C1, C2, C3). Other serial slices were incubated for 1 h at 37°C in Hank's medium alone (C1+H, C2+H, C3+H) or in Hank's medium containing one of the following protease inhibitors (C1+H+I, C2+H+I): diisopropylfluorophosphate (DFP; Aldrich Chemical Company, Milwaukee, U.S.A.) 10 μg/ml; N-α-p-tosyl-L-lysine chloromethyl ketone (TLCK; Sigma, St Louis U.S.A.) 3·7 mg/ml; L-1-tosylamide-2-phenylethylchloromethyl ketone (TPCK; Sigma) 8 mg/ml; phenylmethanesulfonyl fluoride (PMSF; Sigma) 1 mg/ml or aprotinin (commercial preparation: Iniprol) 200 000 IPU/ml. Some skin samples were preincubated prior to cantharide application in aprotinin solution for 2 h at 4°C, to ensure complete penetration of this high molecular weight reagent.

A punch biopsy was taken from the forearm of a healthy volunteer 1 h after topical application of cantharide at the same concentration, and was processed in the same way.

All slices, after fixation in Karnovsky's fixative and post-fixation in osmium tetroxide were embedded in epoxy resin and both large semi-thin sections and ultra-thin sections were prepared.

Possible cantharide cytotoxicity was evaluated using the trypan blue test on trypsinized epidermal cells from skin specimens taken 1 and 2 h after (C1, C2) or without (T1, T2) cantharide application and post-incubation in Hank's medium. Briefly, narrow skin strips (from C1+H, C2+H, T1+H, T2+H) were incubated at 37°C in 0·25% trypsin for 1 h. They were then transferred into culture medium with 10% fetal calf serum to stop the trypsinisation. The epidermis was separated from the dermis with forceps and transferred to phosphate buffered saline for 30 min at 37°C. The suspended cells were filtered through gauze, collected by centrifugation and resuspended in complete medium. Viability of the cells was then determined by trypan blue exclusion.

RESULTS

Light microscopy

Cantharide-induced acantholysis. The results are summarized in Table 1. The earliest changes were noted in the squamous cell layer; an increased density of the perinuclear area, surrounded by a clear zone was observed in the keratinocytes. Some intercellular bridges were still visible (stage A) (Fig. 1a). Subsequent changes consisted of the formation of clefts mainly in the suprabasal layer but also in the granular layer (stage B) (Fig. 1b). Later, bullae appeared progressively (stage C) (Fig. 1c). When acantholysis was complete, the basal cells remained adherent to the basement membrane. The blister roof consisted of the horny layer. The blister cavity contained acantholytic keratinocytes with a well defined nucleolus and a dense cytoplasm (stage D) (Fig. 1c).

Cantharide acantholysis was observed in all subjects (Table 1). The onset and the intensity of

TABLE 1. Cantharide acantholysis seen on light microscopy

Subject no.	Stages of cantharide acantholysis*				
	C1	C1+H	C2	C2+H	C3
1	o	ABC	ABC	C	D
2	o	AB	ABC	C	D
3	o	o	o	ABC	nd
4	o	o	o	AB	nd
5**	o	AB	nd	nd	nd

C1, C2, C3: Direct fixation after 1, 2 or 3 h of cantharide application.

C1+H, C2+H: Fixation after post-incubation for 1 h in Hank's medium.

*Stages: o = No change; A = Increased density of keratinocyte cytoplasm around the nucleus; B = cleft; C = bullae; D = dermis covered by basal cells adherent to the basement membrane.

**Cantharide applied topically to forearm skin 1 h before biopsy.

nd = not done.

the acantholysis were variable from one subject to another and within the same tissue slice. In all cases, no morphological change was noted after 1 h of cantharide application (C1 samples), but post-incubation in Hank's medium resulted in the appearance of a typical acantholytic process (C1 + H samples). Complete acantholysis was always observed 3 h after topical cantharide application (C3 samples), even without post-incubation in Hank's medium. *In vivo* cantharide application gave the same results as those obtained *in vitro* (Table 1).

All controls (T1, T2, T3) were morphologically normal; no acantholysis was observed. Keratinocyte viability, measured, after trypsination, by the trypan blue exclusion test was not significantly different in cantharide pre-treated and untreated epidermis. After 1 or 2 h of cantharide application followed by 1 h post-incubation at 37°C in Hank's medium (C1 + H and C2 + H) the percentages of viable keratinocytes were 49·8 and 42·0 respectively whereas without cantharide application (T1 + H and T2 + H) these values were 53·4 and 42·5, respectively. Thus the high proportion of dead cells must have been due to the trypsination and the long incubation time.

Electron microscopy

Progressive dissolution of the dense plaque leading to the detachment of tonofilaments was the first change observed (Fig. 2a). The released tonofilaments aggregated around the nuclei of the keratinocytes and the attachment plaque completely disappeared (Fig. 2b). At this time, the distance between the desmosomal membranes had decreased and the midlines were not apparent. However, slight thickening of some sections of cytoplasmic membrane at the sites of desmosomal residues persisted, and both desmosomal and non-desmosomal membranes of

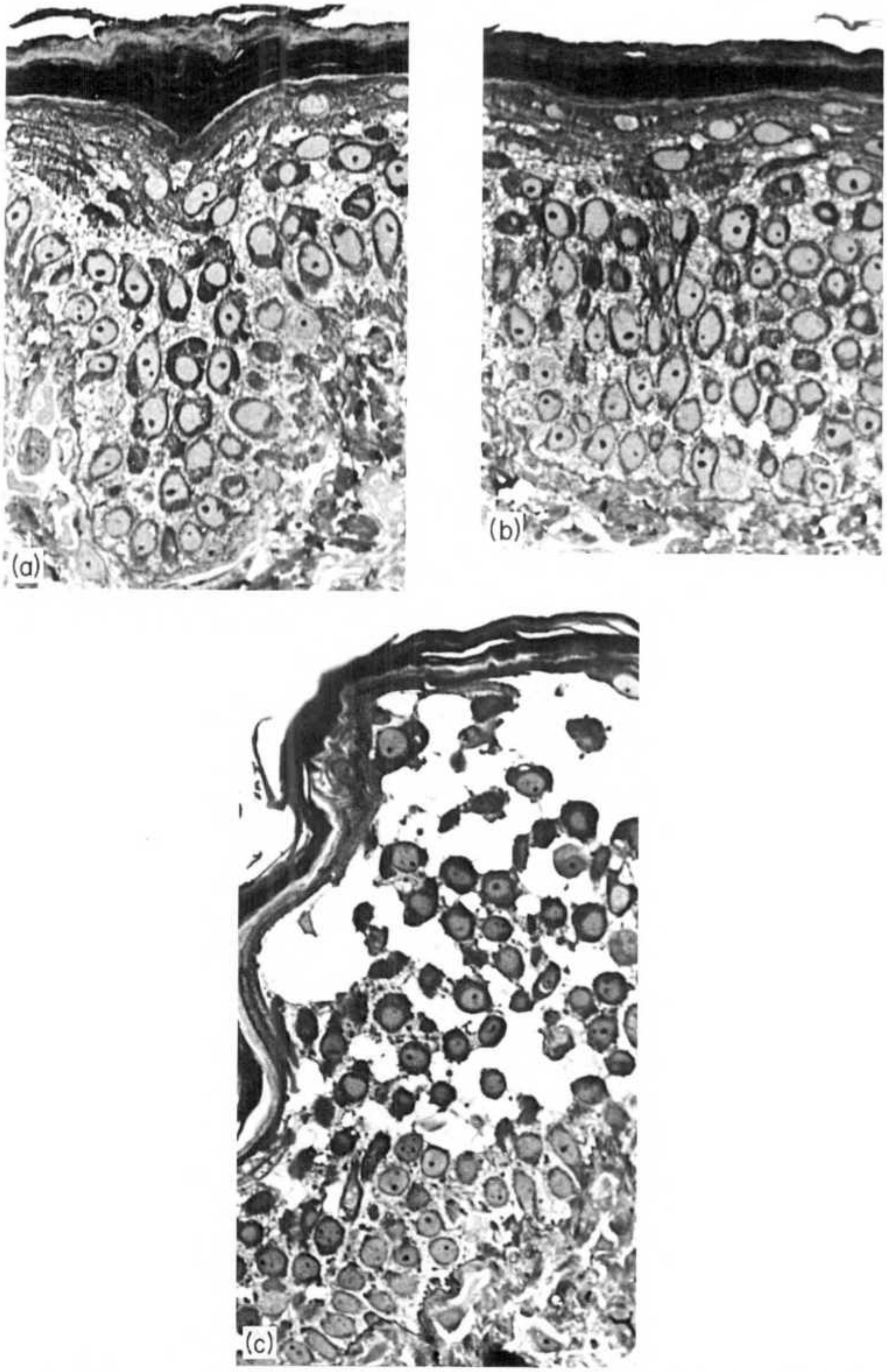

FIGURE 1. Sequence of morphological changes in cantharide acantholysis. (Original × 710). (a) Stage A. Increased density of the perinuclear area surrounded by a clear zone. Some intercellular bridges are still present. (b) Stage B. Cleft formation in a suprabasal location. (c) Stages C and D. Blister formation. The basal cell layer remains adherent to the dermis. The acantholytic keratinocytes have a dense cytoplasm.

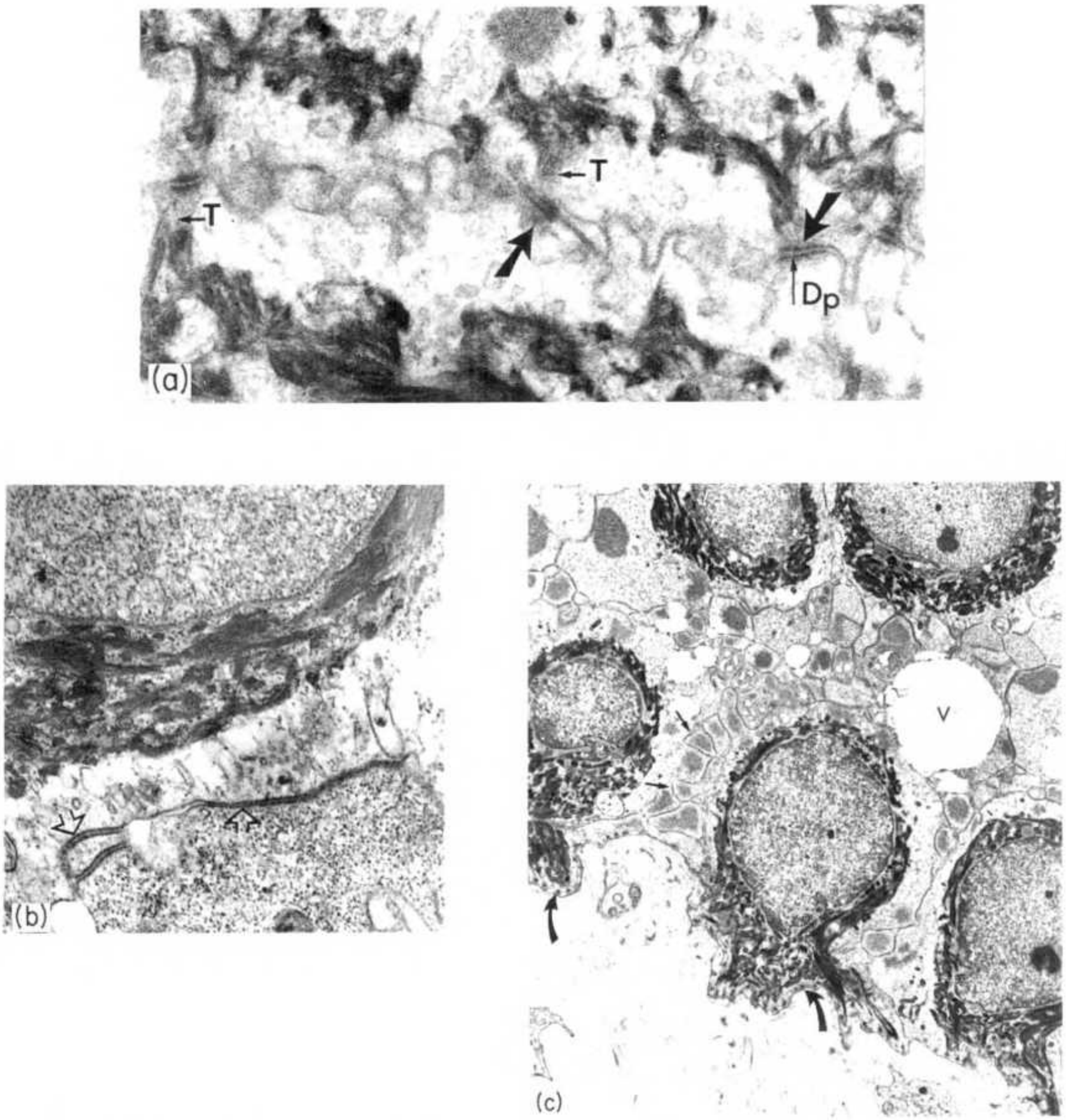

FIGURE 2. Sequence of desmosomal changes in cantharide acantholysis. (a) From left to right, progressive dissolution of the dense plaque (Dp; large arrows) with consequent detachment of tonofilaments (T; small arrows); (Original × 30 000). (b) Complete disappearance of attachment plaque (arrows). Decrease of distance between the desmosomal membranes. Disappearance of the desmosomal midlines. Aggregation of tonofilaments around the nucleus of the keratinocyte. (Original × 30 000). (c) Persistence of slight thickening of some sections of cytoplasmic membrane. Disappearance of desmosomal components with numerous interdigitations of the cell membrane (small arrows). Intercellular vacuolization (V), and conservation of hemidesmosomes (large arrows) (Original × 9450).

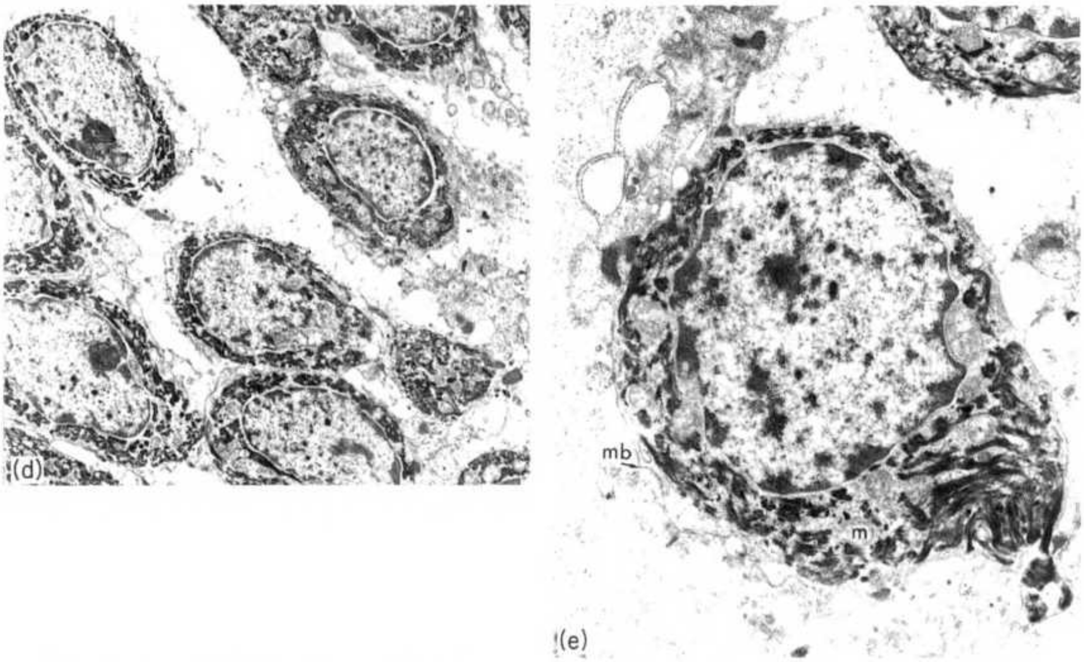

FIGURE 2. (continued). Sequence of desmosomal changes in cantharide acantholysis. (d) Partially acantholytic epidermal cells in blister cavity (Original × 7600). (e) An acantholytic keratinocyte. The cell is relatively intact with a few mitochondria (m) a hyperchromatic nucleus and a cytoplasmic membrane (mb) still present around part of the cell. (Original × 15 600).

TABLE 2. Effect of protease inhibitors on cantharide acantholysis seen on light microscopy

Subject no.	Stages of cantharide acantholysis*						
	C	C+H	C+H+protease inhibitors				
			DFP	TLCK	PMSF	TPCK	Aprotinin
3	o	ABC[a]	o	o	oA	ABC	ABC
4	o	AB[a]	nd	nd	nd	nd	AB
5	o	AB[b]	o	o	oA	AB	nd

C = Direct fixation after cantharide application.

C + H = Fixation after post-incubation for 1h in Hank's medium.

*Stages: o = no change; A = increased density of keratinocyte cytoplasm around the nucleus; B = cleft; C = bullae.

[a] Acantholysis after 2 h of cantharide application.

[b] Acantholysis after 1 h of cantharide application.

nd = not done.

adjacent keratinocytes remained in close contact with many interdigitations (Fig. 2c). At this stage intercellular spaces were not enlarged. Finally, cleft formation and loss of adherence between the epidermal cells occurred as a late event (Figs. 2d and e). In the blister cavity, partially and completely acantholytic cells were found without visible connection between the tonofilaments and the cytoplasmic membrane. Partial loss of the membrane was observed. (Figs. 2d and e).

All these changes were observed in basal, squamous and granular cells. In contrast, neither hemidesmosomes or desmosomes of the horny layer were affected by this process. No morphological change in the desmosome–tonofilament complex was observed in T1, T2, T3 or C1 fragments or in fragments incubated with DFP or TLCK.

Effects of protease inhibitors on cantharide acantholysis

The results are summarized in Table 2. After post-incubation for 1 h in Hank's medium with protease inhibitors we observed that DFP and TLCK completely inhibited cantharide acantholysis, PMSF partially inhibited cantharide acantholysis and aprotinin (even after preincubation) and TPCK did not inhibit cantharide acantholysis.

DISCUSSION

Our results show that the progressive disappearance of the dense desmosomal plaque, leading to the detachment of tonofilaments from desmosomes is the first event in cantharide acantholysis. A primary defect in the desmosome–tonofilament complex was suggested by ultrastructural studies in early lesions of Darier's disease[12–14] and pemphigus foliaceus (PF).[15] In contrast, alteration of desmosomes in pemphigus vulgaris appears at a late stage, secondary to oedema and to widening of the intercellular spaces.[16] Desmosomal alterations induced by cantharide differ totally from the split desmosomes observed in trypsin-dissociated cells[17], in the staphylococcal scalded skin syndrome,[18] and in pemphigus vulgaris acantholysis.[16] Our results also differed with those obtained in skin explants in presence of pemphigus antibodies, in which selective loss of desmosomal components in one cell but not in the adjacent one was shown.[19] In cantharide acantholysis as well as in pemphigus vulgaris and pemphigus foliaceus the retention of intact hemidesmosomes reflects the different properties of this attachment system. The desmosomal changes observed after cantharide acantholysis were not due to the cytotoxicity of cantharide. The cantharide did not alter the viability of keratinocytes as measured by the trypan blue test after trypsinization.

Using various enzyme inhibitors, Stoughton and Bagatell[9] concluded that cantharide acantholysis was probably of an enzymic nature and might be the result of proteolytic processes. In order to study the possible role of specific proteases in cantharide induced acantholysis, we used a method allowing study of the expression and inhibition of the proteolytic activity of these enzymes.[10] The neutral serine protease inhibitors (DFP and TLCK)[20] completely inhibited cantharide acantholysis in our experimental system. Our results using DFP are in agreement with those reported by Stoughton and Bagatell.[9] TPCK, which is an inhibitor of chymotrypsin, but not of trypsin,[21] did not inhibit the cantharide acantholysis. These results demonstrate the involvement of neutral serine proteases in cantharide acantholysis. These specific proteases, released or activated by cantharide, appear to act specifically on the desmosomal plaque. Studying the differential digestion of desmosomal components by pronase, Wolf and Schreiner[22] observed that in epidermis, the attachment plaque was more susceptible to the enzyme than were the tonofilaments. However, pronase is a mixture of several proteases. More interestingly, Shimono and Clementi[23] observed the specific disappearance of the desmosomal plaque in normal oral epithelium of rats treated with a very low concentration of trypsin. Thus, the neutral serine proteases involved in cantharide acantholysis could be trypsin-like. PMSF, which inhibits trypsin and chymotrypsin by reacting with the active serine residue site,[24] only partially inhibited cantharide acantholysis. This could be due to the poor stability of PMSF in aqueous solution, to its activity being heat labile (half life at 25°C, pH 7·5, 35 min),[25] or to the local high concentration of proteolytic enzymes. In addition, we found that aprotinin, an inhibitor of a wide range of serine proteases[26] had no effect on cantharide epidermolysis at 200,000 IPU/ml. This failure has to be compared with the absence of an inhibitory effect observed by Stoughton and Bagatell[9] using SBTI, a well known neutral serine protease inhibitor. These results could be due to the high molecular weight of aprotinin and SBTI preventing efficient diffusion into the epidermis, even after preincubation with thin slices, as in the present study, or injection into the corium.[9] Stoughton and Bagatell[9] also observed that sulphydryl inhibitors and other chemical agents not acting on serine proteases can prevent cantharide acantholysis. Overall, these results suggest that the specificity of the enzymes mediating cantharide acantholysis is similar, but not identical, to that of trypsin, and that the process may involve other enzymes, possibly as part of a biological cascade.

In vivo, desmosomes are known to be labile structures and the mechanism by which the components turn over remains unknown. An endogenous protease such as that described here, which is apparently specific for the dense desmosomal plaque, could play a role in desmosome degradation *in vivo*. Cantharide acantholysis may be a useful model system for the study of such processes.

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The dansyl chloride technique for stratum corneum renewal as an indicator of changes in epidermal mitotic activity following topical treatment

B.D.RIDGE, M.D.BATT, H.E.PALMER AND A.JARRETT*

Beecham Products Research Department, Weybridge, Surrey and * University College Hospital Medical School, London U.K.

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SUMMARY

Using a hypomitotic agent, triamcinolone acetonide, and a hypermitotic agent, retinyl propionate, we investigated the relationship between epidermal mitotic activity and stratum corneum renewal time of topically treated skin as determined by the dansyl chloride staining technique.

Treatment with the base cream resulted in a reduction in renewal time compared with an untreated control site. The predicted increase in renewal time with the hypomitotic agent and reduction with the hypermitotic agent was only observed when daily treatment was commenced 2 weeks prior to and continued after dansyl chloride staining and not when treatment was started after staining.

These results indicate that in order to use cell renewal methods to demonstrate changes in mitotic activity brought about by topical treatments, it is necessary to pre-treat the skin with the test material to establish full epidermal equilibrium at the changed mitotic state before labelling with dansyl chloride. Meaningful claims for effects on cell renewal of specific cosmetic ingredients should only be made after comparison with a base cream treated site, both having been allowed to equilibrate, rather than on the basis of comparison with untreated skin.

Since it was introduced by Jansen *et al.*,[1] the dansyl chloride labelling technique has become widely used as a method for the measurement of stratum corneum renewal time.[2,3] The technique measures the rate of loss of the stratum corneum after application of the dansyl

Correspondence: B.D. Ridge, Beecham Products Research Department, St George's Avenue, Weybridge, Surrey KT13 0DE, U.K.

chloride stain, but this reflects the renewal time only under steady state conditions. It is a non-invasive method that can be applied to human skin, and because of this it has been used on the facial skin of human volunteers to support claims made for cosmetic preparations designed to increase turnover rates in older skins.

The principle is simple and depends on fluorochrome labelling of the full thickness of the stratum corneum. The presence of the fluorochrome can be readily detected under ultraviolet illumination. The time taken for the fluorescence to disappear (renewal time) is assumed to be the time taken for the whole thickness of the stratum corneum to be exfoliated and replaced by new unstained cells from the dividing epidermis.

Under conditions of dynamic equilibrium, where the generation rate at the malpighian layer equals the desquamation rate, Baker *et al.*[4] and Bergstresser *et al.*[5] have argued that the renewal time of the stratum corneum will reflect the rate of epidermal mitotic division. When investigating the effects of a topical treatment it is clearly important to establish a steady rate of epidermal division before measurements are taken. An active test substance will affect the epidermis for an unknown number of days and there will be a continually changing mitotic rate as it adjusts to the stimulus. The whole concept of the method depends on a steady state being achieved in order that stratum corneum renewal times can be related directly to mitotic rates. Measurements made during an unstable period of mitotic activity would invalidate the results obtained by this technique.

If product treatment is started at the same time as dansyl chloride is applied then the new equilibrium state resulting from the treatment may not be reached by the time the stained skin has worn off. This could lead to a failure to detect an effect.

It is also possible that the use of topical treatments might alter the desquamation process and thus decouple the relationship between renewal time and epidermal mitotic activity. Chemical or physical interactions between the various ingredients of the test formulation and the cells of the horny layer could hinder or accelerate their exfoliation. Such effects should be detectable independently of any change in mitotic activity and were examined in the present study using dansyl chloride applied before changes in mitotic activity had become established as a result of treatment.

In order to define the experimental conditions under which a valid extrapolation can be made from stratum corneum renewal time to epidermal mitotic activity for topically treated skin, a proven mitotic inhibitor, triamcinolone acetonide[6,7] and a mitotic stimulator, the propionate ester of vitamin A,[8–10] were examined using the dansyl chloride technique. Each agent was tested in a cream formulation both with and without a pre-treatment period to enable the renewal time of the stratum corneum to be studied at a changed, but steady mitotic rate and also to examine the effects of the test products on the desquamation rate. In each case the effect of the test material on renewal time was compared with that of the base cream without the active ingredient, so that the effect of the other ingredients in the formulation on the desquamation process could be examined and accounted for.

METHODS

Materials

Dansyl chloride (1-dimethylaminonaphthalene-5-sulphonyl chloride) was obtained from BDH Chemicals Ltd. Poole, Dorset, U.K.; Ledercort Cream (triamcinolone acetonide 0·1%) from Lederle Laboratories Division, Cyanamid of Great Britain Ltd. Gosport, Hampshire, U.K.; Aqueous Cream BP from The Boots Company plc, Nottingham, U.K., and Jil Sander Super

Night Treatment (retinyl propionate 3600 IU/g) from Jil Sander Cosmetics, Wiesbaden, West Germany.

Subjects

A total of 88 healthy volunteers (27 men, 61 women) aged between 18 and 57 years took part in the study.

Stratum corneum staining

Dansyl chloride labelling of inner forearm skin was carried out according to the method of Jansen *et al.*[1] A 5% suspension of dansyl chloride in petrolatum was applied to forearm skin on occlusive adhesive patches. After 24 h the patches were removed and excess material swabbed off the skin. The level of fluorescence was assessed daily under UV illumination using an arbitrary scale of 0–8, where 8 = bright fluorescence immediately after staining and 0 = stratum corneum no longer fluorescent.

Regime with no pre-treatment

Each volunteer had two areas on each inner forearm stained with dansyl chloride. One area on each arm was then treated twice daily with either the test formulation or its base, the other two sites acting as untreated controls. Treatment was continued until the skin was no longer fluorescent (i.e. a score of 0).

Regime with pre-treatment

The procedure was the same as that in the regimen without pretreatment except that twice daily treatment was commenced 2 weeks prior to dansyl chloride staining.

Under both regimens the treatments were randomized to the different sites and the effect of the active formulation was compared with that of its base in each subject. Statistical analysis of the data was by Student's paired *t*-test.

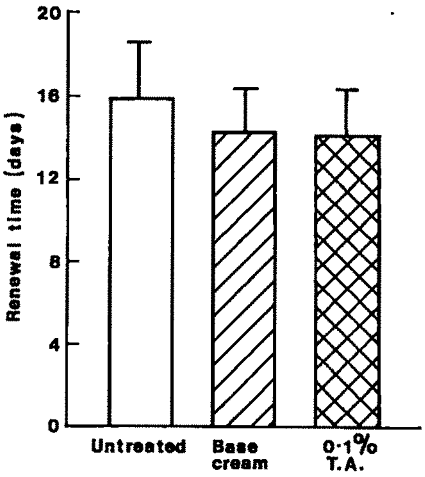

FIGURE 1. Renewal times of stratum corneum treated with 0·1% triamcinolone acetonide (T.A.) or the base cream, without a pre-treatment period. Bars are means ± SD ($n = 20$).

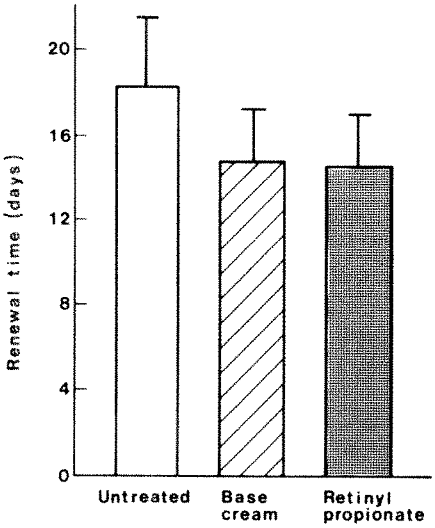

FIGURE 2. Renewal times of stratum corneum treated with retinyl propionate cream or its base without a pre-treatment period. Bars are means ± SD ($n = 20$).

RESULTS

Without pre-treatment

The effect of treatment with triamcinolone acetonide cream and its base on the mean stratum corneum renewal time without a pre-treatment period (measured in 10 males and 10 females) is shown in Figure 1. No difference was detected between the mean renewal times of skin treated with base (14·1 days) or triamcinolone acetonide (14·2 days). Both creams reduced the renewal time compared with that in untreated skin (15·9 days; $P < 0{\cdot}001$).

Similarly, when retinyl propionate cream was tested without pre-treatment (in 13 males and seven females) both the retinyl propionate cream and its base reduced the mean renewal time (14·4 and 14·8 days respectively) compared with that of untreated skin (18·3 days; $P < 0{\cdot}0001$). No significant difference in renewal time was found between the retinyl propionate cream and its base (Fig. 2).

With pre-treatment

When the triamcinolone acetonide cream was compared with its base after 2 weeks pre-treatment (in eight males and 11 females) the mean renewal time for triamcinolone acetonide treated skin was significantly increased (16·5 days) compared with base cream treated skin (15·6 days; $P < 0{\cdot}05$). The renewal time for base treated skin was significantly reduced compared with untreated skin (17·6 days; $P < 0{\cdot}03$) (Fig. 3). Using this pre-treatment regimen the inhibitory effect of triamcinolone acetonide on mitotic activity, compared with that of the base cream, was detected. A typical response to treatment with triamcinolone acetonide cream and its base is illustrated in Figure 4. This photograph, taken under UV illumination (320–400 nm) 11 days after dansyl chloride staining, demonstrates the differences in renewal time for untreated skin, and skin treated with triamcinolone acetonide cream or the base cream.

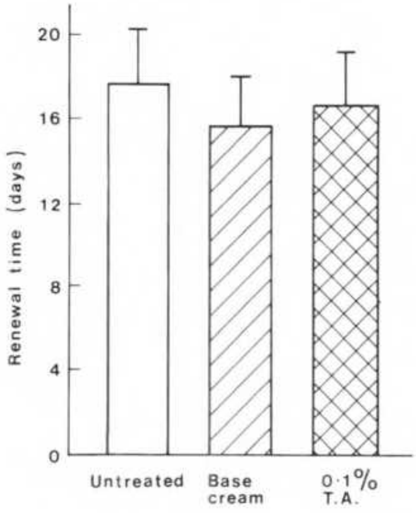

FIGURE 3. Renewal time of stratum corneum treated with 0·1% triamcinolone acetonide (T.A.) or the base cream following 2 weeks pre-treatment.

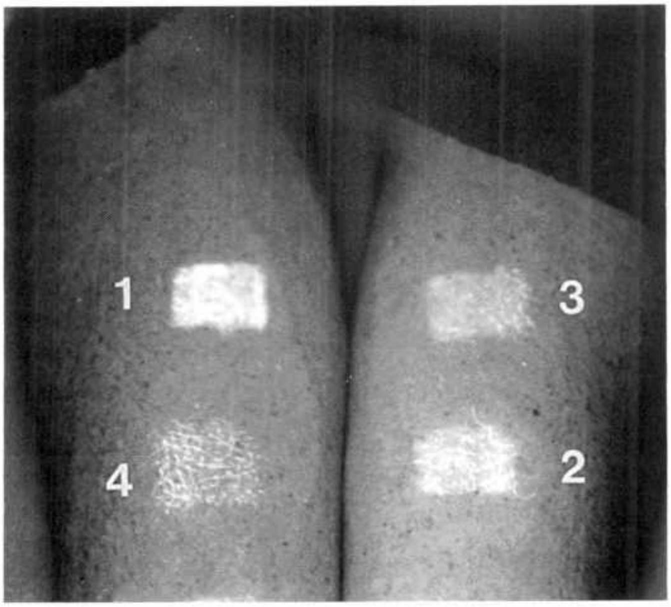

FIGURE 4. An illustration of different levels of fluorescence, 11 days after dansyl chloride labelling, following pre-treatment with 0·1% triamcinolone acetonide cream (2), base cream (3), 0·9% sodium lauryl sulphate solution (4), compared with untreated skin (1).

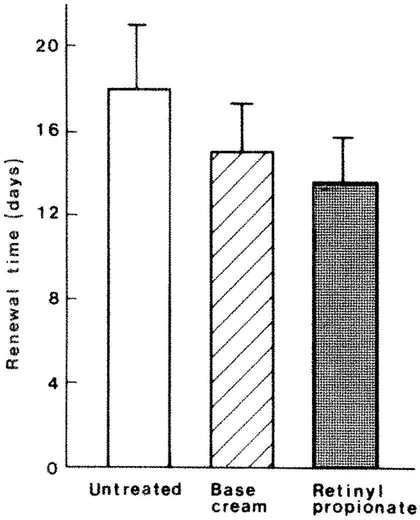

FIGURE 5. Renewal times of stratum corneum treated with retinyl propionate cream or its base following 2 weeks pre-treatment. Bars are means ± SD ($n = 41$).

The remaining site was treated with 0·9% sodium lauryl sulphate solution, as part of a separate investigation into the effects of typical ingredients of skin care formulations, and clearly demonstrates that surfactants accelerate the desquamation process.

When the retinyl propionate cream was tested using a 2-week pre-treatment period (in 41 females) the mean renewal time of skin treated with the retinyl propionate cream (13·5 days) was significantly reduced compared with base cream treated skin (14·9 days; $P < 0{\cdot}0002$). The base cream reduced the renewal time compared with that in untreated skin (17·9 days; $P < 0{\cdot}0001$) (Fig. 5). This demonstrates that the stimulatory effect of retinyl propionate on mitotic activity, compared with the base cream, can be determined by this technique.

DISCUSSION

The time taken for dansyl chloride to disappear from fully stained stratum corneum provides a measure of its renewal time. If these results are to be used to estimate the relative mitotic activity of the basal layer of the epidermis then the technique must respond predictably to agents with a known effect on mitotic activity. Hypomitotic agents should increase the renewal time of the stratum corneum while hypermitotic agents should reduce it. By studying the effects of a hypomitotic and a hypermitotic formulation we sought to investigate the link between stratum corneum renewal and epidermal mitotic activity.

The predicted increase in renewal time with triamcinolone acetonide cream and the reduction with retinyl propionate cream, compared with their base creams, was only observed following 2 weeks of pre-treatment prior to dansyl chloride staining. This would confirm that it is necessary to establish a stable altered mitotic state by a suitable period of pre-treatment, before using the dansyl chloride technique.

In all cases, treatment with the base cream resulted in a reduction in the renewal time compared with untreated skin. Although it is possible that the application of these base creams could increase the mitotic activity of the epidermis, the fact that the effect was observed without pre-treatment suggests an alteration of the desquamation process rather than a change in mitotic activity. Further studies in our laboratory have shown that the surfactants typically used to formulate oil in water and water in oil emulsions produce an immediate increase in the rate of desquamation (Fig. 4). We believe that these surfactants plus the mechanical forces applied to skin during treatment account for the reduction in renewal time observed following base cream treatment.

The dansyl chloride technique depends on the whole depth of the stratum corneum becoming permanently stained with the fluorochrome marker. Histological examination of stained skin and the use of serial adhesive tape strippings have confirmed the findings of Jansen *et al.*[1] that complete stratum corneum staining occurs when dansyl chloride is applied under occlusion. We have also found that 16 h (overnight) exposure is sufficient to stain the full depth of facial stratum corneum, which is in agreement with the findings of Harrison *et al.*[11,12]

The diminishing level of fluorescence was determined daily by expert assessors, who were scoring blind with respect to treatment, until it was no longer visible. In the event of some stained sites reaching an end-point over the weekend it was possible to estimate this point by extrapolation of the daily fluorescence scores.

Attempts to develop a more objective method for the assessment of the level of fluorescence were frustrated by the fine hairs present on the skin which remained fluorescent long after the stain had been lost from the stratum corneum. Preliminary studies in our laboratory indicate that the hairs remain stained for 2–3 months. Methods have been devised to improve the objectivity of measuring fluorescence of the skin[13,14] and we have examined the photographic photometric scale[13] in our laboratory. The procedure was found to be useful for both the training of assessors and also for standardizing the scores of intermediate levels of fluorescence. However, for end-point determinations in the presence of stained hairs we have yet to find an instrument as discerning as the trained human eye. The degree of interference from stained hairs that is routinely encountered is illustrated in Figure 4.

In order to eliminate the problems of regional variation observed by Roberts *et al.*,[2] all comparisons were carried out using randomized sites on the flexor surface of the forearm. Furthermore, because of the reported effects of ageing, and seasonal effects on stratum corneum renewal time, statistical analysis was limited to within study comparisons. This was because the four studies reported here (two active compounds, with and without pre-treatment) used different volunteer panels and were conducted at different times of the year.

As a result of these studies we have defined an experimental regimen which should allow valid conclusions to be drawn regarding mitotic activity using the dansyl chloride technique. The test formulation should be compared with its base, without the active ingredient, both with and without a 2-week period of pre-treatment. If a change in the renewal time is only observed following pre-treatment this can be interpreted as an alteration of the mitotic rate. A change in renewal time without pre-treatment could be indicative of an alteration in the desquamation process.

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Distribution of the antigen in adult linear IgA disease and chronic bullous dermatosis of childhood suggests that it is a single and unique antigen

G.M.POTHUPITIYA, F.WOJNAROWSKA, B.S.BHOGAL* AND M.M.BLACK*

Department of Dermatology, The Slade Hospital, Oxford, and *Institute of Dermatology, United Medical and Dental Schools of Guy's and St. Thomas' Hospitals (University of London), Lisle Street, London, U.K.

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SUMMARY

The tissue distribution and species expression of the antigens in adult linear IgA disease (LAD) and chronic bullous dermatosis of childhood (CBDC) was studied and compared with those of bullous pemphigoid (BP) and epidermolysis bullosa acquisita (EBA) antigens.

Positive sera from five adult LAD patients, 15 CBDC patients, three BP patients and two EBA patients were studied, using skin and mucosal tissues of humans and animals as substrates.

The tissue distribution and phylogenetic distribution of adult LAD antigen and CBDC antigen were the same, suggesting that they are identical diseases. The antigens were both demonstrable only in stratified squamous epithelia and amnion of mammalian species. They were absent from transitional and columnar epithelium.

The EBA antigen was not expressed in pig tissues or human placenta, unlike adult LAD and CBDC antigens. Expression of adult LAD, CBDC and BP antigens was observed in all tissues except bladder, which lacked LAD and CBDC antigens.

These results suggest that the antigens in adult LAD and CBDC are the same and differ from BP and EBA antigens.

Both linear IgA disease (LAD) of adults and chronic bullous dermatosis of childhood (CBDC) are distinct clinical entities characterized by the presence of linear IgA deposits at the dermo-epidermal junction.[1,2] Vesiculobullous skin lesions and mucosal lesions are found in both diseases. In adult LAD, mucosal involvement tends to be commoner and more severe in the

Correspondence: Dr F. Wojnarowska, Department of Dermatology, The Slade Hospital, Headington, Oxford OX3 7JH, U.K.

form of oral and genital ulceration and cicatrising conjunctivitis.[3,4] Both diseases respond to dapsone and sulphapyridine or sulphamethoxypyridazine.

The identity of the antigens in adult LAD and CBDC is unknown. Neither is it known if the antigens in adult LAD and CBDC are the same. Similarly, the expression of these antigens in human and animal tissues has not been systematically studied.

In the present study, we have investigated the tissue expression and phylogenetic distribution of adult LAD and CBDC antigens and compared these with those of the bullous pemphigoid (BP) and epidermolysis bullosa acquisita (EBA) antigens.

METHODS

Human tissues

Samples of skin, conjunctiva, oral mucosa, and rectal and vaginal mucosa were obtained, with permission, from patients undergoing surgical procedures. Placental tissue was obtained from three fresh placentas removed during caesarian section. Other human tissue samples were obtained from a fresh cadaver within 6 h after death.

Animal tissues

Samples of skin and mucosa were obtained immediately after the death of the animals. Tissues from pig, rat, rabbit and toad were studied. All tissues were snap frozen and stored in liquid nitrogen.

Patients' sera

Sera obtained from five patients with adult LAD and 13 patients with CBDC were used for indirect immunofluorescence studies. All these patients had a vesiculobullous disorder, which responded to dapsone or sulphapyridine or other sulphones. The diagnosis had been confirmed by previous direct and indirect immunofluorescence studies, which showed linear IgA deposition at the dermo-epidermal junction. The patients did not have circulating IgG anti-basement membrane zone antibodies.

The sera were diluted 1:5 or 1:10 in phosphate buffered saline (PBS), and pooled adult LAD sera and pooled CBDC sera were also studied.

Sera from three patients with BP (binding to the epidermal side of 1·0 M NaCl split skin) and two patients with EBA were also studied. One of the EBA sera was provided by Dr W.R.Gammon (University of North Carolina, Chapel Hill, U.S.A.).

Normal human sera, at the same dilution, were used as controls, in addition to a PBS control.

Indirect immunofluorescence

Indirect immunofluorescence was performed using a standard technique, on 5 μm cryostat sections. All sera were tested in duplicate on all substrates in at least two separate runs. On bladder and placenta where there were differences in antigen expression, the test was repeated six times to ensure that the results were consistent for all sera.

RESULTS

The tissue distribution and species expression of adult LAD, CBDC, BP and EBA antigens are shown in Table 1.

TABLE 1. Tissue distribution of LAD, CBDC, BP and EBA antigens

		No. of sera showing basement membrane zone fluorescence			
Species	Tissue	LAD ($n=5$)	CBDC ($n=13$)	BP ($n=3$)	EBA ($n=2$)
Human	Skin	5	13	3	2
	Conjunctiva*	5	13	3	0
	Oral mucosa	5	13	3	2
	Bladder (neoplastic)	0	0	0	0
	Vagina	5	13	3	2
	Placenta*	5	13	3	0
	Oesophagus	5	13	3	2
	Stomach	0	0	0	0
	Small intestine	0	0	0	0
	Rectum	0	0	0	0
	Lung	0	0	0	0
	Gall bladder	0	0	0	0
	Kidney	0	0	0	0
Pig	Skin	5	13	3	0
	Conjunctiva	5	13	3	0
	Oral mucosa	5	13	3	0
	Oesophagus	5	13	3	0
	Bladder*	0	0	3	0
	Vagina	5	13	3	0
	Rectum	0	0	0	0
	Small intestine	0	0	0	0
	Kidney	0	0	0	0
	Liver	0	0	0	0
Rat	Skin	5	13	3	0
	Conjunctiva	5	13	3	0
	Oral mucosa	5	13	3	2
	Oesophagus	5	13	3	2
	Bladder*	0	0	3	0
	Rectum	0	0	0	0
Rabbit	Oesophagus	5	13	3	0
	Bladder*	0	0	3	0
Toad	Skin	0	0	0	0

All sera were tested in duplicate on all substrates on two separate occasions and identical results were obtained with all serum samples from each group of patients.

*The staining on these substrates was repeated six times to ensure the consistency of the results.

Tissue expression

The adult LAD and CBDC antigens were present at the basement membrane zone of the skin, including the area surrounding the hair follicles.

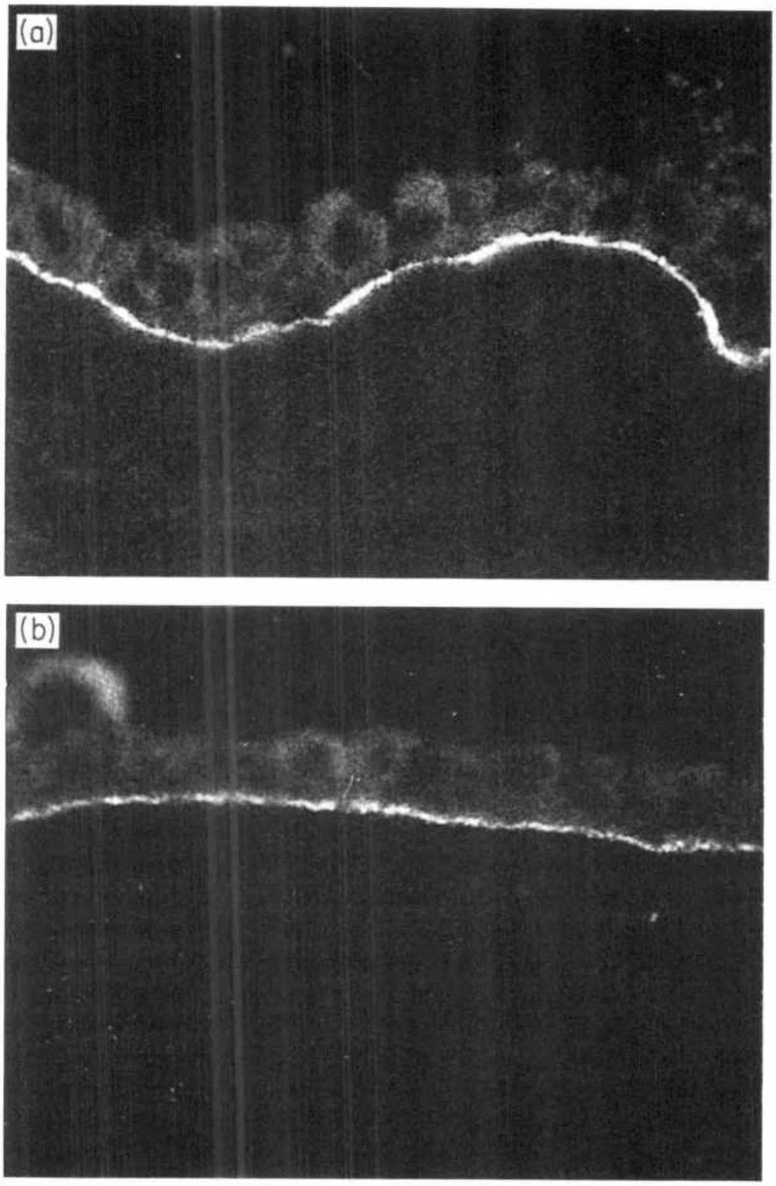

FIGURE 1. Indirect immunofluorescence staining of human placenta showing basement membrane zone fluorescence, (a) with adult LAD serum (original × 25), (b) with BP serum (original × 25).

The antigens were also expressed in the basement membrane zones of many other mucosal tissues characterized by stratified squamous epithelia. They were present in the basement membrane zone of the amnion of human placenta (Fig. 1a); they were absent from the bladder (Fig. 2a), stomach, small intestine, gall bladder, large intestine, rectum, lung, kidney and liver.

Phylogenetic distribution

The adult LAD and CBDC antigens were found in skin and mucosal tissues of humans, and those of pig, rabbit and rat.

On indirect immunofluorescence using sera from adult LAD and CBDC, toad skin showed binding at the basement membrane zone. This was considered non-specific, because the same

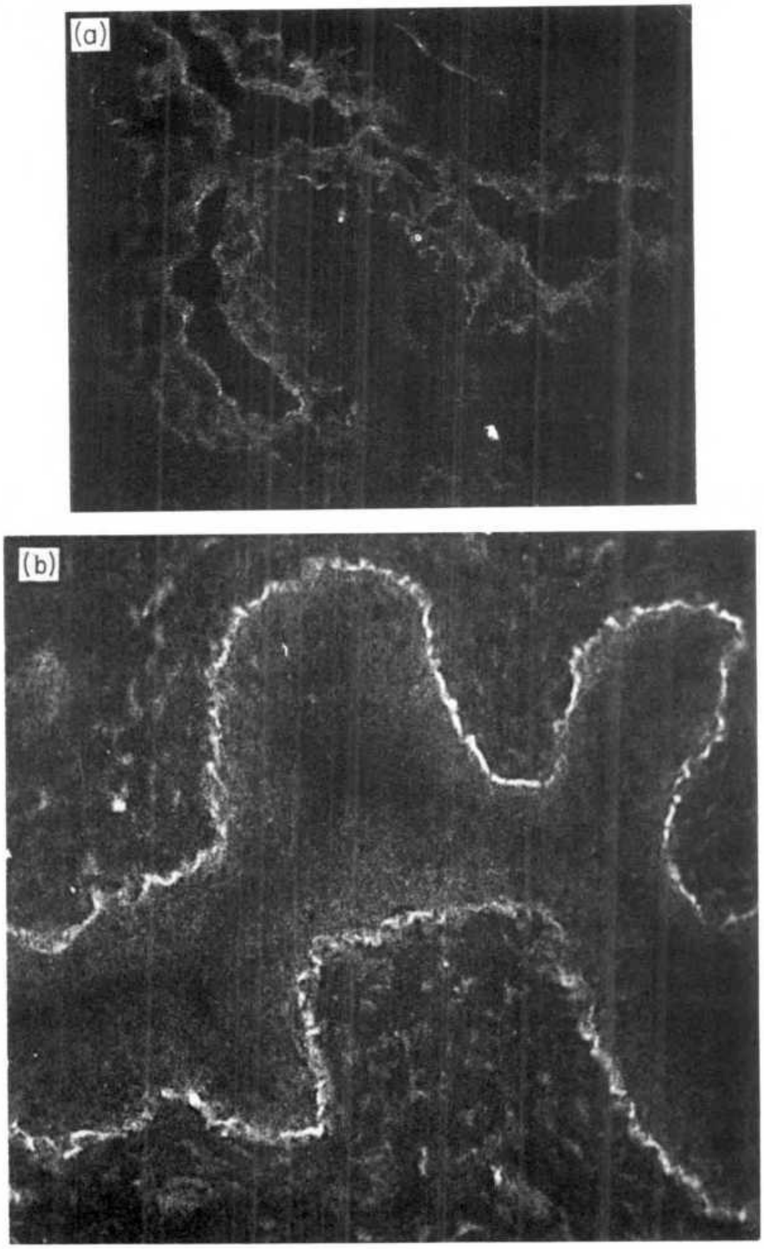

FIGURE 2. Indirect immunofluorescence staining of rabbit bladder, (a) with adult LAD serum showing a negative reaction (original × 25), (b) with BP serum showing basement membrane zone fluorescence (original × 16).

finding was observed with normal human sera. In the previous studies using purified BP IgG antibodies this non-specific binding was not observed.[5]

Comparison of adult LAD and CBDC antigens

No difference was observed between adult LAD antigen and CBDC antigen, either in tissue distribution or in species expression.

Comparison with BP antigen

The distribution of the BP antigen was as originally described by Beutner *et al.*[6] and Robinson *et al*[7] (Fig 1b) with the exception of its absence from post mortem gall bladder and lung.

The antigen of adult LAD and CBDC was absent from transitional epithelia of all normal bladder tissue specimens examined while BP antigen was present in all of these (Fig 2b). Neoplastic human bladder tissue was negative for all three antigens.

Comparison with EBA antigen

The distribution of the EBA antigen was similar to that found in previous studies.[8,9] EBA antigen was absent from all the pig tissues studied. EBA antigen was also absent from human placenta and bladder as previously reported.[9]

DISCUSSION

Adult LAD and CBDC antigens were present in human tissues, and tissues from pig, rat and rabbit. We were unable to demonstrate the antigens in toad skin. Both antigens were found in the stratified squamous epithelia of skin, conjunctiva, oral mucosa, oesophagus and vaginal tissues. In addition, both adult LAD and CBDC antigens were expressed at the basement membrane zone of the amnion of human placenta. The transitional epithelia of all the normal bladder tissue specimens from the species examined were negative for both antigens. Similarly, the columnar epithelia of the bronchi, small intestine, rectum and gall bladder also were negative. In renal tissue, the glomerular and tubular basement membrane zones did not show evidence of antigen expression.

No difference was observed between the antigens of adult LAD and CBDC, either in species expression or tissue distribution.

The antigen in LAD was similar to BP antigen in phylogenetic distribution and expression in stratified squamous epithelia and human placenta. Thus, stratified squamous epithelia and amnion of human placenta are sources of both LAD antigen and BP antigen.[7] Both LAD and BP antigens were absent from the columnar epithelia. In contrast to our findings, the presence of BP antigen has been observed previously in lung and gall bladder.[6] This may reflect heterogeneity in the antibodies or their affinities. However, the LAD antigen was absent from all normal bladder tissue specimens examined, whereas BP antigen was present.

Our split skin studies have shown the localization of adult LAD and CBDC antigens to the epidermal side of the split resembling the reaction pattern of BP antigen.[10,11] However, suction blister studies have revealed that both adult LAD and CBDC antigens can be present in the lamina lucida.[12] Our previous immunoelectron microscopic studies have shown deposition of antibodies from adult LAD and CBDC patients at the same site as EBA antibodies.[13] The findings from these studies, together with our present study, suggest that adult LAD and CBDC antigens differ from BP antigen in location as well as tissue distribution, despite the similarity in phylogenetic distribution. The lack of cross reactivity of an antibody considered specific to this antigen with the BP antigen is in accordance with this suggestion.[14]

The EBA antigen was absent from all the pig tissues examined, unlike adult LAD and CBDC antigens. Thus, the species distribution of EBA antigen differs from that of adult LAD and CBDC antigens. There were also differences in the tissue distribution of adult LAD and CBDC antigens and EBA antigen. Although all three antigens occurred in most stratified squamous epithelia, EBA antigen was not identified in human conjunctiva or vaginal epithelium. Similarly, this antigen was absent from rat skin, although it has been demonstrated previously in rat skin and the skin of other rodents.[9] This could be explained by species differences in antigen expression or variable antibody affinity.

Split skin studies using EBA sera showed a reaction on the dermal side of the split,[10] whereas adult LAD and CBDC antigens were present on the epidermal side, suggesting a difference between the two antigens. Together with the findings of the present study, this suggests that the antigens of adult LAD and CBDC differ from the antigen in EBA in location, tissue expression and species distribution.

All these observations suggest that the antigen in adult LAD is different from both BP and EBA antigens. However, no difference has been detected between adult LAD and CBDC antigens and it seems likely that both these clinical entities share a common and unique antigen. Further studies using immunoblotting techniques are required to confirm this.

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Low-dose cyclosporin A in severe psoriasis. A double-blind study

TH.VAN JOOST, J.D.BOS,* F.HEULE AND M.M.H.M.MEINARDI*

Department of Dermato-Venereology, University Hospital Rotterdam Dijkzigt, Erasmus University, Rotterdam and * Department of Dermato-Venereology, Academisch Medisch Centrum, University of Amsterdam, The Netherlands

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SUMMARY

Twenty patients with severe plaque psoriasis were selected to receive either low-dose cyclosporin A (CyA) or placebo (CyA vehicle) in a double-blind randomized trial at two centres. Within 4 weeks the mean reduction in the Psoriasis Area and Severity Index (PASI) in 10 patients receiving CyA (mean dose 5·5 mg/kg/day) differed significantly from the mean reduction in 10 patients receiving placebo. In eight patients given placebo a switch to CyA therapy resulted within 4 weeks in a mean reduction in PASI of 90%. In a total 15 out of 18 patients given CyA (83%) (mean dose 5·6 mg/kg/day) there was an improvement of ≥75% in PASI within 4 weeks. In a 2-month tapering off phase a lower mean CyA dose (3 mg/kg/day) was effective in maintaining the reduced PASI scores in seven of nine patients. Four out of five CyA treated patients who entered a post-treatment observation phase had a relapse (PASI score ≥50% of score at baseline) after a mean interval of 6·5 weeks.

The most important side-effects were mild reversible hypertension in 5 of 18 patients (28%), and reversible elevated serum creatinine levels in 7 of 18 patients (39%). We consider that further studies are justified in severe chronic psoriasis to establish suitable regimens for maintenance of remission in psoriasis with low-doses of CyA or a combination of CyA with other anti-psoriatic agents.

Cyclosporin A (CyA) exerts its effects by selectively influencing the functional activation of lymphocyte subsets.[1,2] CyA has received increasing attention in clinical dermatology in general[3,4] and as a possible useful drug in the treatment of psoriasis in particular. Occasional case reports originally appeared on the effectiveness of CyA in inducing remission of concomitant psoriasis when used for other conditions, such as arthritis[5] or after organ transplantation.[6,7] Open trials then demonstrated that low-dose CyA (3–5 mg/kg/day) could be effective in severe psoriasis which was resistant to conventional therapy.[8–10] A controlled

Correspondence: Professor Th.van Joost, Department of Dermato-Venereology, Academisch Ziekenhuis Rotterdam, Dr Molewaterplein 40, 3015 GD Rotterdam, The Netherlands.

double-blind study in which a much higher dose of CyA was used (14 mg/kg/day), confirmed that the drug improved psoriasis significantly.

We have carried out a controlled double-blind study of low dose CyA in 20 patients with severe chronic plaque psoriasis resistant to other therapy.

METHODS

Selection of patients

Twenty patients, seven males and 13 females (aged 20–70 years), (10 patients from each of two centres) entered the trial after giving their informed consent. Criteria for inclusion were: unequivocal histological diagnosis of chronic plaque psoriasis, severe recalcitrant progressive disease, duration of disease at least 10 years, and resistance to conventional therapy (topical steroids, PUVA, oral methotrexate or retinoids singly or in combination). Patients were excluded if they were pregnant or had an acute uncontrolled bacterial, viral or fungal infection, malignancy or a history of malignancy, impaired renal function (serum creatinine > 100 μmol/l), bilirubin or liver enzymes more than twice the upper limit of the normal range, uncontrolled hypertension (ages 18–50 blood pressure $> 150/95$ mm Hg, ages 50–60 blood pressure $> 160/100$ mm Hg), epilepsy or malabsorption syndrome, if they abused drugs or alcohol or were being treated with nephrotoxic drugs (e.g. aminoglycosides or non steroidal anti-inflammatory drugs) or drugs known to affect the pharmacokinetics of CyA (ketoconazole, erythromycin, phenytoin, phenobarbitone, rifampicin, isoniazid or carbamazepin). Other important criteria for exclusion were: treatment with methotrexate, retinoids or PUVA therapy during the month preceding the trial and use of topical anti-psoriatic agents within 2 weeks of the start of the trial. Clinical evaluation was carried out using the psoriasis area and severity index (PASI).[12] The PASI increases in units of 0·1 from 0·0: no lesions, to 72·0: complete psoriatic erythroderma of the severest possible degree. On entering the study all patients had a PASI of 20 or above.

A double-blind study was carried out in parallel on 10 patients in each centre.

Phase 1

A double-blind comparison of CyA with placebo was carried out for 4 weeks. The patients were kept in hospital for this phase of the study. Cyclosporin A (Sandimmune®) or placebo (vehicle consisting of olive oil and polyethylated oleic glycerin) was given in two equal daily doses (at breakfast and at supper), 1 ml of solution contained 100 mg CyA. Daily doses were adjusted on the basis of body weight; subjects weighing up to 59 kg received 3 ml, 60–80 kg 4 ml and more than 80 kg 5 ml. The dose of CyA in individual subjects was reduced by 1 ml if there was: (a) a rise in serum creatinine of more than 50% above the baseline (baseline $\leqslant 100$ μmol/l),[13] (b) a rise in serum potassium above the upper limit of the normal range, (c) an increase in serum bilirubin or liver enzymes above twice the upper limit of the normal range, (d) hypertension unresponsive to antihypertensive therapy or (e) trough blood levels of CyA above 900 ng/ml. If the abnormal values did not normalize within 2 weeks the CyA dose was further reduced by 1 ml. If this was ineffective or in subjects who developed a significant infection, the test medication was withdrawn. Non-responders were defined as patients who had a reduction in PASI score of less than 50% at week 4 compared to their score at the start of the trial. Their test medication was stopped and the code broken. Patients who had received placebo were switched to CyA-treatment in an open out-patient trial using the same dose schedule of CyA as in phase 1.

Phase 2

The patients who were considered responders at the end of week 4 continued to receive the test medication double-blind, and this was tapered off over a period of 2 months (weeks 5–12). The medication was reduced in each subject at 2-weekly intervals on the basis of the dose being taken at the end of week 4, and the CyA doses are listed in Table 1. The code was broken for the remaining patients at the end of week 12. If the PASI score increased again and reached a value equal to or above 50% of score before therapy (baseline) the disease was considered to have relapsed. If a relapse occurred, the test medication was stopped and alternative therapy was given.

Phase 3. Three month post-treatment observation period.

The patients who had received CyA in phase 2 entered phase 3 if they consented to receive no anti-psoriatic medication. The PASI was measured monthly. Patients were excluded if a relapse occurred or if they opted to receive anti-psoriatic therapy.

During phase 1 creatinine and potassium were measured at weekly intervals. Blood pressure, bilirubin and liver enzymes, urine analysis and full blood count were measured every 2 weeks. Trough blood CyA concentrations 10–14 h after the last dose of CyA were measured using the Cyclosporin RIA-Kit (Sandoz Ltd, Basel, Switzerland). Measurements were made weekly in phase 1 and at 2-week intervals in phase 2.

Statistical analysis was performed using the Mann-Whitney U test to compare the changes in PASI scores (score at end of week 4 minus score at baseline) in the CyA and placebo groups.

RESULTS

The doses and PASI scores in patients receiving CyA are summarized in Figure 1 and Table 2. The CyA dosage was reduced when the blood level exceeded 900 ng/ml. This was necessary in Patient 10 in week 2 and Patient 2 in week 3. However, these appeared to be peak levels and thus, were not due to an overdose of CyA. No consistent relationship between the clinical response and trough serum CyA levels could be found.

TABLE 1. Tapering off schedule for the dosage of CyA during weeks 5–12 of the trial (phase 2) on the basis of individual doses at the end of week 4

	Dose of CyA ml/day*		
At end of week 4 (phase 1)	3	4	5
In weeks 5 & 6	2	3	4
In weeks 7 & 8	2	2	3
In weeks 9 & 10	1	2	2
In weeks 11 & 12	1	1	1

* 1 ml contains 100 mg CyA.

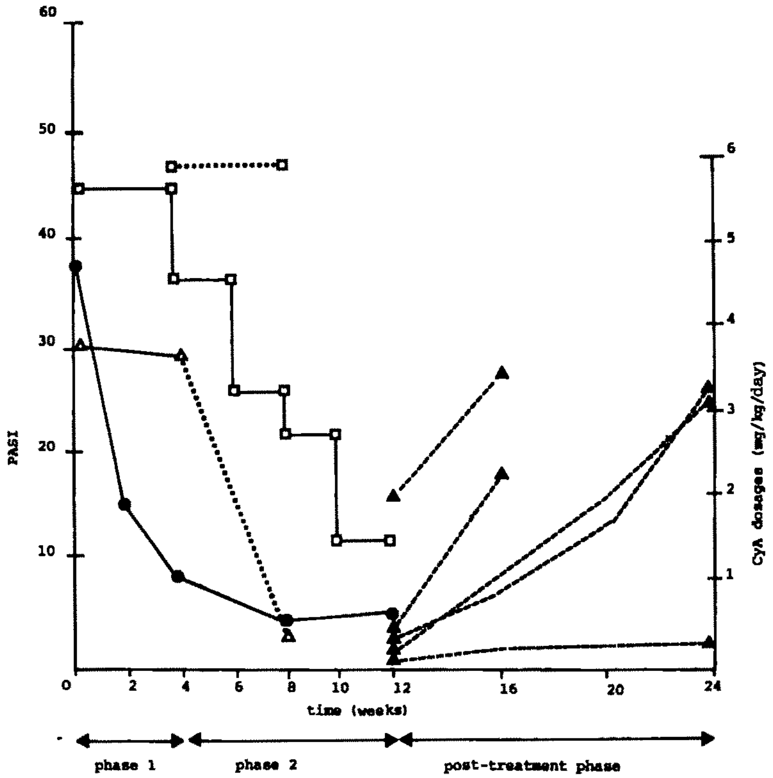

FIGURE 1. Mean CyA dosages and mean PASI scores in patients receiving CyA or placebo. □——□: mean dose of CyA (mg/kg/day) in patients receiving CyA at the start of the trial ($n = 10$, during weeks 1–4; $n = 9$, during weeks 5–12); ●——●: mean PASI in patients receiving CyA at start; △——△: mean PASI in patients receiving placebo at start ($n = 10$); □······□: mean dosage of CyA (mg/kg/day) after switch of placebo group to CyA ($n = 8$); △······△: mean PASI in placebo group after switch to CyA ($n = 8$). ▲----▲: Individual PASI scores in five patients during phase 3.

Phase 1

At the end of phase 1 (week 4), 11 patients of the group of 20 were classified as non-responders and the code was broken. All, except one (Patient 6) had received placebo. On breaking the code in the responders (at the end of week 12) altogether 10 patients (mean PASI at baseline 36, mean age 53 years) had received CyA and 10 patients (mean PASI at baseline 30, mean age 46 years) had received placebo. The mean percentage reduction in the PASI score at the end of week 4 in the group treated with CyA (72%) was significantly greater than the mean percentage reduction in the placebo group (3%) ($P < 0{\cdot}001$). In four patients in the placebo group worsening of the psoriasis was seen during phase 1. During phase 1 the mean dose of CyA in the CyA treated group was 5·5 mg/kg/day with a mean trough blood level of CyA of 519 ng/ml (excluding blood levels of CyA > 900 ng/ml). Patient 6, considered a non-responder at week 4 had a substantial fall in the PASI score when the dose of CyA was increased to 6·7 mg/kg/day for 4 weeks (PASI score 3·9, percentage reduction in PASI 79%). Patient 7 weighed 93 kg, but had accidentally received a lower dose of CyA (4 ml/day = 4·3 mg/kg/day) than intended. However, at week 4 this patient showed a reduction in PASI of 67%.

TABLE 2. Reduction in PASI scores in 10 patients with severe psoriasis treated with cyclosporin A during phase 1

					Percentage reduction in PASI compared with baseline		PASI in phase 3		
Patient	Sex	Age (years)	Body weight (kg)	PASI score at baseline	Week 4 ($n = 10$)	Week 12 ($n = 9$)	Week 16	Week 20	Week 24
1	M	64	66	29·7	80%	99%	2·1	2·1	2·1
2	M	61	64	34·0	64%	34%(R)†	Relapse in week 12		
3	M	41	59	42·8	77%	91%	8·3	13·1	27·1(R)†
4	F	66	77	42·2	83%	84%	Dropped out		
5	M	62	71	51·5	76%	88%	Dropped out		
6	M	56	59	21·4	18%*	...	...	...	...
7	F	61	93	52·5	67%	71%	27·8(R)	...	...
8	M	25	86	43·0	90%	97%	2·6	15·3	26·4(R)
9	M	29	64	20·0	80%	76%	16·5(R)	...	...
10	M	66	63	28·4	81%	96%	Dropped out		
Mean		53	70	36·5	74%**	82%			

* Patient 6 considered a non-responder.

** Excluding Patient 6, mean percentage reduction in PASI was 79%.

† (R) = relapse. PASI score ≥ 50% of baseline value.

Phase 2

The mean percentage reduction in PASI (compared with baseline in the nine patients in this phase of the trial) was 82% at week 12, compared with 79% at week 4 (Patient 6 excluded). All nine patients had received CyA. In one patient (Patient 2) a relapse was observed during week 12. In all nine patients a substantial improvement occurred in psoriatic nail lesions. The mean CyA dose during this tapering off phase (weeks 5–12) was 3 mg/kg/day with mean trough CyA blood levels of 317 ng/ml. During the last 2 weeks (weeks 11 and 12), the mean trough blood concentration of CyA was 131 ng/ml ($n = 8$) (mean CyA dosage 1·4 mg/kg/day; $n = 9$).

Phase 3

At the start of this phase of the trial three of the remaining eight patients insisted on receiving further CyA therapy or alternative anti-psoriatic therapy. Thus, only five patients entered phase 3 of the trial (Fig. 1). In two patients a relapse was seen at week 16 (1 month after CyA was stopped), two patients had relapsed at week 24, and one patient maintained a PASI of 2·1 (baseline PASI 30·2).

Switch to CyA therapy in the placebo group

Two patients preferred to receive alternative treatment. The mean percentage reduction in PASI in the remaining eight patients who received CyA treatment (mean dose 5·7 mg/kg/day) was 90% after 4 weeks when compared with PASI at the end of 4 weeks on placebo.

Side-effects

In none of the patients was the CyA dose reduced because of clinical or biochemical (kidney or liver function) side-effects. The clinical side-effects in the total of 18 patients receiving CyA

TABLE 3. Percentage reduction in PASI scores in all patients treated with CyA

% Reduction in PASI	No. of patients After 4 weeks CyA ($n=18$)	After 12 weeks CyA ($n=9$)
91–100	4 (22%)	4 (45%)
75–90	11 (61%)	3 (33%)
51–74	2 (11%)	1 (11%)
25–50	1 (6%)*	1 (11%)‡
0–24	0 (0%)	0 (0%)
Mean dosage of CyA	5·6 mg/kg/day	3 mg/kg/day

* Patient 6, see text and Table 2.
‡ Patient 2, see text and Table 2.

were mild hypertension (five), aching muscles (five), fatigue (two), mild hypertrichosis (two male), headache (one), mild gynaecomasty (one male), tremor (one), gastrointestinal upset (one) and development of verruca vulgaris (one). In none of the CyA treated patients was altered liver function detected. In seven of the 18 patients (39%), a slight rise in serum creatinine was observed. This was less than 40% above their respective individual baselines. In all patients the serum creatinine level rapidly returned to normal when the CyA dose was reduced (phase 2) or stopped.

DISCUSSION

Generally it can be concluded that for a therapeutic result the oral dose of CyA is more important than the trough blood levels. An almost complete remission of recalcitrant psoriasis was seen within 4 weeks in four out of 18 patients (22%) with a low mean dose of CyA (5·6 mg/kg/day), while in 15 (83%) a significant improvement (reduction in PASI of ≥75%) was found. These results confirmed that low-dose CyA was superior to placebo. In view of the relatively minimal side-effects CyA represents an advance in the short-term treatment of psoriasis.

A difference was found in the mean percentage reduction in PASI score after 4 weeks of CyA treatment in the group of patients receiving CyA initially (72%) and in the group that had received placebo previously (90%). This difference was probably due to the varying sensitivity of psoriasis to CyA in individual cases. In one patient (Patient 6) there was a reduction in PASI score of less than 50%. This patient, however, had an excellent response when the CyA dose was increased. The results of our study, together with the experience of other authors,[11] appear to indicate the existence of a subgroup of poor responders to CyA among patients with plaque form psoriasis. Our experience has indicated that low-dose CyA (5 mg/kg/day) is of less or no value in patients with the active guttate form of psoriasis, especially when this is triggered by bacterial infections (Van Joost Th. *et al.*, unpublished observations). A case of recalcitrant psoriasis pustulosa (Von Zumbusch-type), which responded to a much higher dose of CyA, has been reported recently.[14]

Despite the limited number of CyA-recipients (nine patients) studied in the tapering off phase

(phase 2) and the apparent variation in response to CyA, some preliminary conclusions with regard to the efficacy of low-doses can be drawn. In this phase, despite a lower mean CyA dosage (3 mg/kg/day) a higher mean percentage reduction in the PASI score (82%) was observed.

In 3 patients (Patients 1, 8 and 10) who received CyA during weeks 9–12 at doses between 2 and 3 mg/kg/day, a further reduction in their PASI was noted. In a recent open trial,[10] by increasing the dose, clearance of psoriasis was observed after 2–12 weeks (mean 7·4 weeks) at doses of CyA between 1 and 5 mg/kg/day (mean 3·3 mg/kg/day). These data[10] and our present results appear to demonstrate that 3 mg/kg/day represents an effective CyA dose in certain subgroups of patients with chronic plaque psoriasis.

Relapse of psoriasis occurred in most, but not all patients after the withdrawal of CyA, but the timing and rate of relapse differed between individuals. In phase 3 of the trial, considerable problems were faced in maintaining a genuinely treatment-free regimen. Three patients insisted on receiving additional treatment. After CyA treatment was stopped, four patients had a relapse after 4 weeks and two after 12 weeks (mean time to relapse 6·5 weeks). One patient still showed an almost complete remission of psoriasis 3 months after stopping CyA treatment.

In none of the patients were adjustments in the CyA dose required for important clinical or biochemical side-effects. In three patients temporary anti-hypertensive therapy was required. One patient's blood pressure normalized spontaneously in the tapering off phase of the trial.

CyA may interfere with T cell functions, by interference with interleukins, in particular with interleukin-2.[15] The striking response of severe psoriasis to low-dosages of CyA could be considered a powerful argument for an essential role of T cells in psoriasis.[16–18] An additional immunological explanation for the effectiveness of CyA in psoriasis might be that the drug interferes with the antigen presenting capacity of dendritic cells,[19,20] especially in view of the presence *in vivo* of relatively large numbers of dendritic cells bearing Langerhans cell and interdigitating cell immunophenotypes in psoriasis.[18] It has been reported that CyA does not inhibit epidermal cell growth.[21] However, abnormal keratinocyte proliferation in psoriasis induced by epidermal growth stimulating factors liberated by inflammatory cells might be inhibited indirectly by CyA due to its effects on T cells.[22,23] Of interest are observations indicating that even after a short period, 3 to 7 days, of high-dose CyA treatment, the epidermal mitotic activity in psoriatic skin is decreased significantly.[11] This observation fits in with our finding that within 2 weeks of treatment with low-dose CyA, reductions in PASI score of 50% or more can be obtained.

We have suggested that low dose CyA might be of use in limited courses for the treatment of severe episodes of psoriasis ('crisis intervention').[8] In that study, it was shown that slightly increased serum creatinine levels normalized again after withdrawal of CyA. However, additional monitoring of the glomerular filtration rate is needed in further prospective studies in larger groups of patients to arrive at an optimal regimen for CyA therapy in psoriasis. If we can be certain about the lack of serious side-effects, low-dose CyA therapy could have an important place in maintaining clearance of or improvement in severe psoriasis for longer periods.

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Relationship between body weight and blood pressure and some metabolic parameters in psoriatic patients

ANDREA PESERICO, G.ZANETTI,* S.PADOVAN, PATRIZIA BERTOLI,
C.VELLER FORNASA, R.CIPRIANI, G.B.AMBROSIO,* S.ZAMBONI,*
AND A.PAGNAN*

Clinica Dermatologica, Istituto di Medicina Clinica, and *Clinica Medica I, Universita' di Padova, Italy

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SUMMARY

The relationship between body weight and blood pressure, total serum cholesterol, triglycerides, uric acid and glucose was investigated in 203 psoriatic patients in comparison with 904 healthy controls. In the psoriatic patients, these parameters were clearly related to their body weight. Those psoriatic patients of normal weight exhibited lower mean values of all the parameters compared with overweight psoriatic patients while they did not differ from control subjects of normal weight. Our results suggest that only overweight psoriatic patients exhibit the metabolic abnormalities frequently reported in the literature, while psoriatic patients of normal weight do not differ from the general population in this respect.

There have been many reports of an increased prevalence of hypertension,[1,2] dyslipidaemias[3–5] diabetes mellitus,[6–8] and hyperuricaemia[9] in psoriasis. However, the pathogenesis of these conditions in psoriatic patients has not yet been elucidated. In the present study we have measured systolic and diastolic blood pressure, total serum cholesterol, triglycerides, glucose and uric acid in psoriatic patients and normal controls and related these to body weight.

METHODS

Two hundred and three psoriatic patients (107 males and 96 females), consecutively referred to our clinic from 1983 to 1985, were studied. The mean age was 42 years (range 20–59 years) in the males and 43 years (range 20–59 years) in the females. A control group of 904 randomly selected and apparently healthy subjects (453 males and 451 females), from the same geographical area, who had been enrolled into an epidemiological study on the prevalence of 'risk factors' for

Correspondence: Professor Andrea Peserico, Clinica Dermatologica, Università di Padova, via Cesare Battisti 206, 35100 Padova, Italia.

arteriosclerosis was also studied. The mean age of the controls was 42 years (range 20–59 years) in the males and 42 years (range 20–59 years) in the females.

Body weight, height and body mass index were measured in all subjects. Those subjects whose body weight was more than 10% above their ideal body weight, calculated according to Lorentz's index,[10] were considered overweight. Total serum cholesterol, serum triglycerides, and serum uric acid and glucose were measured in an SMAC 24 autoanalyser using standard methods. Systolic and diastolic blood pressure were measured using a Riva Rocci sphygmomanometer, in the lying position, after a 15 min rest period. An unpaired Student's t-test was used for statistical analysis of the data.

RESULTS

In the psoriatic patients 57·9% of the males and 51·1% of the females were overweight while in the control subjects 52·8% of the males and 54·9% of the females were overweight. Results for body mass index, basal serum glucose, total cholesterol, triglycerides and uric acid, and diastolic and systolic blood pressure in psoriatic and control subjects are listed in Table 1. In the male psoriatic patients the mean body mass index, and serum triglycerides and uric acid levels were significantly higher than in controls, while mean total serum cholesterol, and diastolic and systolic blood pressure were lower, but not significantly so. In female psoriatic patients the mean body mass index, and serum triglycerides and uric acid levels were higher than in the controls, but only in the two latter parameters was the difference statistically significant. In the female psoriatic patients the mean total serum cholesterol, and diastolic and systolic blood pressure were lower than in the controls, but only the difference in blood pressure was statistically significant. Measurements of these parameters in psoriatic patients and controls who were overweight and in those who were of normal weight are listed in Tables 2 and 3. The overweight psoriatic patients had higher mean levels of all the parameters as compared with psoriatic patients of normal weight and these differences were statistically significant for total cholesterol ($P < 0{\cdot}05$), triglycerides ($P < 0{\cdot}01$), systolic blood pressure ($P < 0{\cdot}01$) and uric acid ($P < 0{\cdot}01$) in the males (Table 2) and for total cholesterol ($P < 0{\cdot}01$), triglycerides ($P < 0{\cdot}01$), uric acid

TABLE 1. Measurements of body mass index, metabolic parameters and blood pressure in psoriatic and control subjects. Values are means ± SD

	Males		Females	
	Psoriatic (n = 107)	Control (n = 453)	Psoriatic (n = 96)	Control (n = 451)
Body mass index (kg/m^2)	26·25±3·6	25·12±3·12	25·76±5·12	25·03±4·39
Basal serum glucose (mg/dl)	92·3±15·1	91·4±21·2	89·6±27·2	86·5±13·4
Total serum cholesterol (mg/dl)	202·7±45·8	210·5±47·1	197·7±45·3	202·1±43·9
Serum triglycerides (mg/dl)	156·3±95·3	126·8±98·5	115·3±79·6	92·1±57·8
Serum uric acid (mg/dl)	6·1±1·3	5·7±1·4	4·9±1·3	4·3±1·0
Diastolic blood pressure (mmHg)	83·8±7·7	85·1±11·8	81·8±9·9	85·1±12·8
Systolic blood pressure (mmHg)	134·5±16·7	137·5±19·8	132·5±19·9	137·1±20·2

TABLE 2. Measurements of metabolic parameters and blood pressure in male psoriatics and controls in relation to body weight. Values are means ± SD

	Psoriatics		Controls	
	Overweight ($n=62$)	Normal weight ($n=45$)	Overweight ($n=239$)	Normal weight ($n=214$)
Basal serum glucose (mg/dl)	93·6±14·4	90·2±15·2	92·4±18·1	89·5±34·3
Total serum cholesterol (mg/dl)	210·5±49·1	193·0±37·6	225·5±48·4	205·0±11·6
Serum triglycerides (mg/dl)	180·2±103·2	123·3±70·7	143·2±44·1	106·9±68·9
Serum uric acid (mg/dl)	6·5±1·5	5·7±1·0	6·1±1·4	5·5±1·1
Diastolic blood pressure (mmHg)	84·8±8·5	82·2±6·8	88·2±11·9	80·8±11·3
Systolic blood pressure (mmHg)	138·3±17·8	129·9±13·3	142·9±20·5	133·8±17·2

TABLE 3. Measurements of metabolic parameters and blood pressure in female psoriatics and controls in relation to body weight. Values are means ± SD

	Psoriatics		Controls	
	Overweight ($n=49$)	Normal weight ($n=47$)	Overweight ($n=248$)	Normal weight ($n=203$)
Basal serum glucose (mg/dl)	96·1±36·2	85·3±17·5	88·4±17·7	83·1±38·1
Total serum cholesterol (mg/dl)	208·6±51·6	178·1±49·9	218·2±45·1	196·7±45·5
Serum triglycerides (mg/dl)	136·9±88·7	92·8±62·1	107·1±64·6	79·4±49·4
Serum uric acid (mg/dl)	5·3±1·2	4·5±1·2	4·7±1·1	4·2±1·2
Diastolic blood pressure (mmHg)	84·7±9·2	78·6±9·7	88·6±12·1	77·6±11·3
Systolic blood pressure (mmHg)	138·6±19·4	126·3±18·7	147·5±22·7	130·3±17·8

($P<0{\cdot}01$), and systolic ($P<0{\cdot}01$) and diastolic ($P<0{\cdot}01$) blood pressure in the females (Table 3). The same trend is also evident in the control group in that all parameters, both in males and females, were significantly higher in the overweight subjects as compared with subjects of normal weight with the sole exception of basal glucose. The overweight male psoriatic patients had significantly higher mean values for triglycerides ($P<0{\cdot}01$) and significantly lower mean values for total serum cholesterol ($P<0{\cdot}05$) and dyastolic blood pressure ($P<0{\cdot}01$) compared with the overweight male controls, while none of the other parameters differed significantly between the two groups. The overweight female psoriatic patients had significantly higher mean values for trygliceride ($P<0{\cdot}05$) and uric acid ($P<0{\cdot}01$) and significantly lower values for diastolic blood pressure ($P<0{\cdot}05$) and systolic blood pressure ($P<0{\cdot}01$) compared with the overweight female controls. In the psoriatic patients of normal weight the mean values for all the parameters, with the sole exception of total serum cholesterol in females, did not differ significantly from the means in the controls of normal weight.

DISCUSSION

An increased incidence of hypertension,[1,2] dyslipidaemias,[3–5] diabetes mellitus[6–8] and hyperuricaemia[9] have been reported in psoriasis. It is well known that such pathological conditions are more frequent in obesity, but few workers have investigated the correlation between obesity or overweight and the above mentioned pathological conditions in psoriatic patients.[2,3,8–10] In the present study there was no significant difference in the proportion of overweight individuals in the psoriatic and control groups. The psoriatic group however showed a greater degree of overweight. The body mass index was higher in psoriatic patients compared with controls and this difference was statistically significant in the males ($P < 0{\cdot}01$). When the psoriatic patients are considered independently of their body weight it can be seen, in both sexes, that our results agree with previous studies with respect to an increase in the mean serum levels of triglycerides[3–5] and uric acid,[9] and a decrease of the mean serum level of total cholesterol.[5,12,13] However, alteration of these parameters in the psoriatic patients was clearly related to their body weight. The psoriatic patients of normal weight had, in both sexes, lower mean levels of all the parameters measured, compared with the overweight psoriatic patients, while they did not differ significantly from the control subjects of normal weight; the only exception being the mean cholesterol levels which were significantly lower in psoriatic females of normal weight than in controls.

Our results suggest that it is only overweight psoriatic patients who exhibit the metabolic abnormalities which have been reported, while psoriatic patients of normal weight do not differ from the general population.

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A survey of the social and psychological effects of psoriasis

B.RAMSAY AND MYRA O'REAGAN*

Dermatology Department, Royal Victoria Infirmary, Newcastle upon Tyne, U.K. and * Statistics Department, Trinity College, Dublin, Republic of Ireland

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SUMMARY

One hundred and four consecutive patients with psoriasis who were being treated in two Dublin dermatology hospital units answered a questionnaire. Social and emotional morbidity was present for many patients despite access to modern treatment facilities. Fifty five percent of patients had never experienced a complete remission from their psoriasis. A large percentage of patients avoided common social activities, for example swimming and sports; 50% felt that psoriasis had inhibited their sexual relationships and 11% of patients said they would avoid having children in case their offspring should develop the condition. Thirty-six per cent felt the hospital doctor spent most of the consultation time writing prescriptions and 59% of patients had never been given an explanatory pamphlet or book about psoriasis.

To help patients to come to terms with what is for many a lifelong condition, greater efforts should be made to improve the communication at consultation, and provide more patient education.

Much of the care of our patients with psoriasis, in the hospital setting, involves devising and implementing topical and systemic therapies. Despite major improvements in these therapies and more frequent remissions, many patients still have recurrent or chronic disease activity.

Earlier surveys have shown some of the social and emotional morbidity that accompanies chronic psoriasis.[1,2] In the present report we outline the factors associated with this morbidity, and the sources of patient knowledge about psoriasis, and also aim to highlight the wider spectrum of problems that face the patient with psoriasis.

METHODS

One hundred and four patients with psoriasis (51 males, 53 females; mean age 35 years, range 16–74 years) currently attending two Dublin dermatology hospital units were seen consecu-

Correspondence: Dr B.Ramsay, Dermatology Department, Royal Victoria Infirmary, Newcastle upon Tyne, NE1 4LP, U.K.

tively. Fifty percent of the patients were married and, of those who were single, 3% were divorced or separated and 3% widowed. All five socio-economic groups were equally represented. The mean age at onset of psoriasis was 21 years, mean duration 13·6 years, and all patients had had psoriasis for at least 6 months prior to the study.

A detailed history of each patient's illness was taken by the same doctor (B.R.). To compare the responses of the patient to the extent of their disease, each patient was examined and the plaque distribution mapped on body diagrams and assessed as percentage body area affected. Disease extent was classified as: mild, < 10% affected; moderate, 11% to 40%; or extensive, > 41%.

A questionnaire was completed by the patient, who was given a room aside from the consultation area and as much time as necessary to complete it.

The initial questions were to assess how often, if ever, common social activities were avoided because of psoriasis. There was a series of questions reviewing the patient's emotional perceptions of their condition and its effects on social interaction with non-sufferers. Patients were asked about the influence of psoriasis on their choice of career, and time lost from work because of psoriasis in the previous 12 months. Patients outlined what they felt to be the worst factor in having psoriasis, and were asked to record the time spent on treating their skin.

To ascertain which parts of the medical services or society were fulfilling an educative role for psoriatic patients, further questions assessed possible sources of patient knowledge on their condition, which source they found most informative and whether they had ever received any explanatory written material about psoriasis. The level of knowledge about their condition was studied and opinions of the role fulfilled by the hospital doctor.

RESULTS

History and examination

A history of psoriasis in first degree relatives was given by 48% of patients. Fifty five percent had never had a complete remission from their condition since its onset. Of the patients who had had a complete remission, the mean period was 8 months, range 1 month–2 years. Of the 104 patients, 87·5% had chronic plaque psoriasis, 8·7% had guttate psoriasis and 3·8% had palmoplantar psoriasis.

The extent of the psoriasis is shown in Table 1. The scalp, elbows and knees were the most frequently affected areas; 37% had facial psoriasis and 34% had hand psoriasis; 25% of patients had psoriatic nail changes and 30% had genital lesions. Some patients had both hand and nail psoriasis. The majority of patients (80%) had been treated with topical tar, dithranol and UV radiation; 19% had had photochemotherapy and less than 4% had had retinoid or methotrexate treatment. Sixty four percent had had previous hospital admissions and of these patients 55% had had only one admission; 65% were out-patients at the time of the survey and 35% were in-patients. Over a third of the patients were in-patients because the Dublin Dermatology Units

TABLE 1. Disease extent in 104 psoriatic patients studied

Percentage of body surface affected	Percentage of patients
< 10% (Mild)	24
11–40% (Moderate)	38
> 41% (Extensive)	38

provide care for many rural patients who would be otherwise unable to avail themselves of treatment. The patients seen consecutively were not necessarily new patients. In Dublin Dermatology Units new and review out-patients are booked for 20 min and 10 min consultations respectively. Alternative medicine had been used in the recent past by 20% of patients. The largest proportion (15%) had attended faith healers—the remaining 5% had taken advice and treatment from homeopathic or acupuncture practitioners. None of the patients felt the alternative therapies had made any difference to their condition.

Patient questionnaire

Social activities. Large numbers of patients avoided swimming and sunbathing because of their psoriasis (72% and 60%, respectively). Although many of these patients had moderate or extensive disease, 46% of those with mild disease also avoided swimming and sunbathing. Thirty four percent avoided attending a hairdresser's and half of these patients were those with extensive disease. Forty percent avoided playing sports because of their psoriasis and the majority of these patients had moderate or extensive disease. Communal baths and showers were avoided by 64%. Only 14% avoided dancing—presumably because affected sites could be covered. A percentage (11·5) avoided leaving their own homes because of their psoriasis. Of the total 11·5% did not avoid any of the seven social activities listed while 70% of patients avoided at least four of these activities.

Clothing. Twenty five percent of patients avoided buying clothes altogether because of their psoriasis; 64% avoided buying short-sleeved summer clothes, summer dresses or shorts. Dark-coloured clothes were avoided by 33% because of the scaling from the scalp.

None of these patterns of avoidance was restricted to patients with moderate or extensive disease; 41% of those with mild disease avoided summer shorts or dresses.

Emotional perceptions. Fifty seven percent of patients felt people stared at them because of their psoriasis. Similar percentages (55% or more) felt non-sufferers regarded their condition as contagious, felt 'untouchable' or 'like a leper' to other people or were embarrassed when questioned about their psoriasis by non-sufferers, and 56% regarded their bodies as 'unclean' because of their psoriasis.

These feelings were equally common in the mild, moderate and extensive disease groups.

Sexual relationships. In answer to the question: 'Do you feel having psoriasis has inhibited your sexual relationships?' 50% of patients answered in the affirmative; 65% of these had plaques in the genital area. Table 2 shows that there is a direct relationship between patients' perceptions of psoriasis inhibiting their sexual relationships and disease extent.

TABLE 2. Inhibition of sexual relationships in relation to extent of psoriasis

	% of patients			
Do you feel having psoriasis has inhibited your sexual relationships?	Mild	Moderate	Extensive	Patients with genital lesions
Yes	37·5	47·5	61·5	65
No	62·5	52·5	38·5	35

TABLE 3. Influence of psoriasis on choice of career in relation to extent of disease

Has psoriasis influenced the choice of career you have pursued?	% of patients		
	Mild	Moderate	Extensive
Yes	8·3	12·8	17·5
No	91·7	87·2	82·5

TABLE 4. The relationship between extent of psoriasis and time off work in the previous 12 months

How much time have you had to take off in the last 12 months because of psoriasis?	% of patients			
	Mild	Moderate	Extensive	Total
0 weeks	62·5	64·9	30·6	51·5
1–8 weeks	25·0	32·4	50·0	37·1
9–52 weeks	12·5	2·7	19·4	11·3

Those with plaques in the genital area (39%) were almost twice as likely to have inhibition of their sexual relationships as patients without genital psoriasis.

Eleven percent of the patients said they would avoid having children because of their fear that their offspring would develop psoriasis.

Choice of career and time off work because of psoriasis. The majority (86·4%) of the patients studied did not feel that psoriasis had influenced their choice of career. Of the remaining patients, 14% felt psoriasis had adversely affected their career decisions, and had actually discontinued careers because of the appearance of their psoriasis to non-sufferers.

The greater the disease extent the more difficulties in choice of career arose for patients with moderate and extensive psoriasis (Table 3).

Although over 50% of patients did not lose any time from work, 37·1% had been off work for 1–8 weeks in the previous 12 months and 11·3% for even longer—some of these had been off work for the whole of the previous year.

Half of those patients with extensive disease had been off work for 1–8 weeks (Table 4).

The worst factor. Patients were asked to indicate what they felt to be the worst factor about having psoriasis and the results are shown in Table 5; 86% felt that the problems they had experienced with psoriasis would be considerably lessened if the general public were more aware and knowledgeable about the disease.

Time spent on treatments. Table 6 shows the time spent on treatments according to disease extent; 87% of patients in all three categories (mild to extensive) treated their skin at least once a day.

TABLE 5. The worst factor about having psoriasis

Factor	% of patients
General appearance of the skin	48
Itching	31
Flaking of the skin	17
Awkward or messy treatment programmes	4

TABLE 6. Time spent daily on treatments in relation to disease extent

How long does it take you to treat yourself at home?	% of patients		
	Mild	Moderate	Extensive
10 min	62·5	50·0	32·5
½ hour	33·3	42·5	47·5
An hour or longer	4·2	7·5	20·0

Over 90% of patients with mild or moderate disease spent 10–30 min daily on their treatment; 20% of those with extensive disease took 1 h per day on their treatment. Thirty percent of patients stated that moderate or potent topical corticosteroids were a routine part of their therapy.

Sources of knowledge about psoriasis. Forty-eight percent found that their general practitioner had been a helpful source of information compared with 63% who found their hospital doctor helpful; 33% felt the hospital nurse had contributed to their knowledge. Television, radio, newspapers and magazines all had low scores as helpful sources of knowledge. The Psoriasis Association (Ireland) was helpful for only 4% of patients. When asked which was the most helpful source, 52% listed the 'hospital' in contrast to 10% who found the general practitioner the best source. However, 59% of patients had never been offered an explanatory pamphlet or book about psoriasis. Twenty eight percent thought psoriasis was curable (cure defined as being cleared forever by treatment). Seven percent believed their condition to be contagious.

Opinion of hospital doctors. Thirty six percent felt that the hospital doctor spent most of his or her time in the consultation writing prescriptions. The remainder indicated that their hospital doctor did spend time listening to their questions or attempting to educate them about psoriasis.

DISCUSSION

We consider that the group of patients studied reflects the broad spectrum of psoriatic patients seen in dermatology hospital in-patient and out-patient services. Sixty four percent had mild or moderate disease and this is reflected in the fact that only 19% had had photochemotherapy and very low percentages had had oral or systemic treatment.

As has been indicated in previous studies,[1-3] our survey has shown that large numbers of psoriatic patients continue to experience substantial social distress. This is partly expressed in avoidance of many common social activities, and this was not resticted to patients with moderate or extensive disease, but included many with mild disease. This also applied to the 11·5% who had such social phobia that they found it difficult to leave their homes because of their appearance. This shows the degree to which even mild or moderate disease can interfere with normal social functioning.

Over half the patients had negative feelings about their condition and also about the way other people viewed them. There is evidence that psoriasis can have an inhibitory influence on sexual relationships and this appears to worsen with increasing disease extent. Medical staff need to be more aware of the possible difficulties experienced by the sexually active patient. Treatment therefore, should not be solely aimed at clearance of the rash, but should enable the patient to feel comfortable enough in the consultation to broach such problems. It also requires a willingness on the part of the medical staff to develop new consultation skills and to listen to and discuss sensitive issues.[4]

That the disease can have a profound effect on patients is further reflected in the number (11%) who said they would avoid having children in case they should develop psoriasis.

It is encouraging that psoriasis did not appear to influence choice of career in many patients. Fifty percent of patients had not lost time from work in the past year. However, many married women who completed the questionnaire commented that it did not include the time that they had lost from household duties during in-patient or out-patient treatment.

Understandably, for 48% of patients the worst factor about psoriasis was the general appearance of their skin. However, it is interesting that for 31%, pruritus was the worst factor. This has an important therapeutic implication because if some of these patients were in a Koebner reactive state, trauma by scratching could perpetuate their condition.[5] As their physicians, we should pay more attention to this symptom and treat it accordingly.

Considerable time is spent by patients on their treatment as out-patients. It should be of some concern that 30% of patients are still using moderate or potent corticosteroids topically on a regular basis and may be at risk of the known local and systemic side effects of these preparations. It is also of interest that 20% utilized different forms of alternative medicine.

Although 52% of patients found the 'hospital' to be their most helpful source of knowledge about psoriasis, it was disturbing that 59% had never received any written advice about their condition. It has been previously well documented that patient education needs written as well as verbal reinforcement.[6] This lack of written information may explain why over a quarter of patients (27%) thought their condition was curable.

Dissatisfaction with the dermatological consultation may account for the number of patients (36%) who regarded their hospital doctors as prescribers only. This echoes the findings of previous studies.[2]

The general practitioner was listed as being the most helpful source of knowledge about psoriasis for less than 10%. The responsibility for patient education rests with hospital dermatology departments and patient association groups. The central educative role of the dermatologist has been outlined previously.[6]

Much of the hospital-based management of psoriasis revolves around the application of physical remedies, and research. Whilst this is vital and laudable, the social and psychological effects of the condition on the patient often can be forgotten or actively ignored, because they are too time-consuming or difficult to deal with during the consultation. The possibilities of a curative therapy for psoriasis are still some way off and we are often faced with patients who have chronic disease with short term remissions. It is, therefore, important that the hospital dermatology departments should be continually evaluating whether they are responding to all the needs of their patients. Day care centres, patient instruction sheets, education groups and films explaining the myths and realities of psoriasis may all help.[8] However, the lead must be taken by the dermatologist in the basic but potentially dynamic setting of the consultation. Helping the patient with psoriasis to learn to live with their condition is a challenging and important part of our role as dermatologists.

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The microbial colonization of inflamed acne vulgaris lesions

J.P.LEEMING, K.T.HOLLAND AND W.J.CUNCLIFFE*

University Departments of Microbiology and *Dermatology, University of Leeds, Leeds, U.K.

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SUMMARY

Seventy one papules in the early stages of inflammatory development were isolated from acne vulgaris affected skin and their content of micro-organisms characterized. The progress of lesions prior to excision was monitored by tracing an area of the upper back onto a transparent acetate sheet. This template was used the next day, and in some cases after 3 days, to identify inflamed lesions of less than 1 day and 2–3 days duration. These were biopsied, and pilosebaceous units isolated by micro-dissection, homogenized and microbial populations studied by viable counting and microscopy.

Propionibacteria colonised 68% of '1 day' duration lesions and 79% of '3 day' duration lesions; staphylococci colonized 19% and 32% respectively and *Pityrosporum* spp. (*Malassezia furfur*) were found in 52% and 68%. Although the prevalence of each microbial group was higher in the more chronic lesions, these differences were not statistically significant. The microbial profile of inflamed lesions was similar both qualitatively and quantitatively to non-inflamed lesions studied previously. These results call into question the role of micro-organisms as the initiators of inflammation in acne vulgaris.

The presence of micro-organisms in pilosebaceous units is one of several factors believed by many to be important in the pathogenesis of acne vulgaris,[1] but the extent and nature of microbial involvement has proved difficult to establish. The observation that micro-organisms are not universally present in comedones suggests that they are not responsible for the primary events in the development of acne vulgaris lesions, namely comedogenesis.[2,3] However, it is still a widely held view that micro-organisms, particularly *Propionibacterium acnes*, are important in the pathogenesis of the inflammatory processes of acne.[4,5,6] Although many recent investigations have attempted to identify microbial factors of potential importance as inflammatory mediators, relatively few studies have addressed the more fundamental question of the incidence and abundance of micro-organisms in inflamed lesions. This is probably due in part to the difficulty in interpreting data obtained from lesions subject to specific and non-specific immunological activity. This is a particular problem in pustules and long-standing lesions. Papules, especially those which have been inflamed for only short periods, are more likely to retain the microbial flora present when inflammation was initiated, but are often impossible to sample by non-invasive techniques. The present study was undertaken to investigate the

Correspondence: Dr J.P.Leeming, Department of Microbiology, Bristol Royal Infirmary, Bristol, BS2 8HW U.K.

microbiology of papules in their early stages of development, using pilosebaceous units isolated by micro-dissection of biopsy material, after soaking in calcium chloride.

METHODS

Location of lesions

Subjects studied were volunteer acne vulgaris patients (age range 13–39) attending the dermatology clinic at Leeds General Infirmary. None had received antimicrobial treatment for at least 4 weeks. The progress of acne lesions was monitored by tracing an area of the upper back onto a transparent acetate sheet. This template was used the next day to identify papules of less than 1 day duration (referred to as '1 day' lesions). Some of the patients were asked to return after a further 2 days in order to locate papules of 2–3 days duration ('3 day' lesions). The papules studied were palpable lesions with erythema of up to 5 mm diameter.

Isolation of lesions

One lesion of interest was excised from each patient using a 3 mm biopsy punch (Steifel). Biopsies were soaked in 1 mol/l $CaCl_2$ for 2 h at 4°C. Pilosebaceous units were then isolated by micro-dissection. Although several units were normally isolated from each biopsy, follicles at the centre of the erythema usually exhibited comedo-like distention of the duct, which simplified their identification. Units which showed no swelling compared with normal pilosebaceous units were not processed, due to the difficulty in defining such units as papules even if they appeared to be the centre of inflammation. Units which had visibly lost the integrity of the duct wall were also discarded. Results from lesions in which pus-like exudate was observed during dissection were excluded from analyses of data because of the possible detrimental effect of phagocytic polymorphs on microbial viability. Papules conforming to all these criteria were isolated from approximately half the '1 day' biopsies and a third of '3 day' biopsies (the more chronic lesions had more often lost ductal integrity and were more likely to contain pus-like exudate).

Microbiological assessment

Isolated lesions were homogenized in phosphate-buffered 0·1% (v/v) Triton X-100 solution (pH 7·9). Micro-organisms were isolated, enumerated and identified by standard methods as described previously.[7] A minimum of 13 colony forming units per papule could be detected. Each sample was also examined microscopically to substantiate the viable counts. Three 0·1 ml aliquots were dried onto a 12 mm diameter well of a multispot microscope slide (C.A.Hendley, Essex, U.K.). Films were heat fixed and Gram-stained and 40 3×10^{-4} cm^2 fields were examined; yeast cells (presumed to be *Pityrosporum* spp.) were counted and the presence of bacterial cells was noted. The sensitivity of this procedure was approximately 400 cells per papule.

Statistical analysis

Statistical analyses were performed as recommended by Sokal and Rohlf.[8] The G test (used to test the independence of frequency data) is closely analogous to the chi-square test.

RESULTS

Fifty two '1 day' and 19 '3 day' papules conforming to the definitions given above were analysed. These isolated lesions were morphologically similar to non-inflamed comedones examined previously.[3] The bacterial groups most frequently isolated were staphylococci and propionibacteria. Micrococci and aerobic coryneform bacteria were less frequently found and never constituted a major population (always < 100 organisms per papule). Microscopic examinations substantiated the viable counts to the extent that bacteria were never observed in homogenates from which negative cultures were subsequently obtained (although this did occur in several pustules examined).

In Figure 1 the distributions of bacterial population densities in papules are compared with data obtained from normal follicles and non-inflamed comedones in previous studies.[3,7] These distributions were all bimodal. The higher lognormal distributions were assumed to be composed of genuinely colonized follicles. The lower populations displaying a Poisson-like distribution are probably non-resident organisms which became associated either with the mouth of the follicle before dissection or with the outside of the follicle during dissection. Such contamination of micro-dissected units by organisms originating from other follicles or the skin

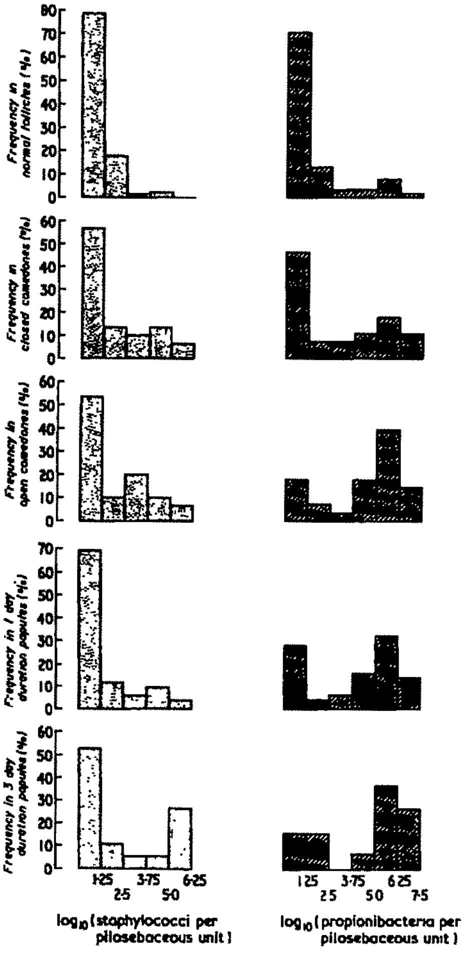

FIGURE 1. Frequency distributions of bacterial population sizes in pilosebaceous units at various stages of acne vulgaris lesion development.

TABLE 1. Colonization of pilosebaceous units by micro-organisms at different stages of acne vulgarus lesion development

	Normal follicles	Closed comedones	Open comedones	'1 day' duration papules	'3 day' duration papules
Staphylococci					
Prevalence*	7/140 (5%)	10/30 (33%)	11/29 (38%)	10/52 (19%)	6/19 (32%)
CFU/unit**	$2\cdot2 \times 10^3$	$8\cdot5 \times 10^3$	$5\cdot9 \times 10^3$	$1\cdot6 \times 10^4$	$4\cdot3 \times 10^4$
	$4\cdot6 \times 10^2$–$1\cdot1 \times 10^4$	$1\cdot8 \times 10^3$–$4\cdot1 \times 10^4$	$1\cdot0 \times 10^3$–$3\cdot4 \times 10^4$	$3\cdot1 \times 10^3$–$8\cdot6 \times 10^4$	$4\cdot0 \times 10^3$–$4\cdot6 \times 10^5$
Propionibacteria					
Prevalence	23/138 (17%)	13/28 (46%)	21/28 (75%)	34/50 (68%)	15/19 (79%)
CFU/unit	$8\cdot0 \times 10^4$	$1\cdot2 \times 10^5$	$2\cdot9 \times 10^5$	$2\cdot7 \times 10^5$	$5\cdot5 \times 10^5$
	$2\cdot5 \times 10^4$–$2\cdot6 \times 10^5$	$2\cdot1 \times 10^4$–$2\cdot1 \times 10^5$	$1\cdot1 \times 10^5$–$7\cdot4 \times 10^5$	$1\cdot2 \times 10^5$–$6\cdot0 \times 10^5$	$1\cdot1 \times 10^5$–$2\cdot8 \times 10^6$
Pitrosporum spp.					
Prevalence	16/126 (13%)	20/29 (69%)	21/27 (77%)	27/52 (52%)	13/19 (68%)
Any group: Expected prevalence (calculated from figures above)				44/50 (88%)	18/19 (96%)
Observed prevalence				45/50 (90%)	19/19 (100%)

*No of units colonized/no sampled, and percentage.

**CFU = colony forming units (viable counts); values are means and 95% confidence limits.

surface is inevitable using the procedures described. Inspection of the data showed that few papules contained between 100 and 1000 bacteria of a particular type. This allowed a simple numerical differentiation of papules considered to be colonised from those containing only superficial populations (or none at all). This division was made at 200 organisms per papule. For consistency, the same value was used to divide other follicle types into colonized and non-colonized units. The proportions of each follicle type colonised, according to his criterion, are listed in Table 1. Statistical analysis (G test) demonstrated that there was a highly significant difference between the proportions of normal follicles and lesions colonised by propionibacteria and staphylococci (both $P < 0{\cdot}001$). There were no statistically significant differences in bacterial colonization rates amongst the four different lesion types compared (open and closed non-inflamed comedones and '1 day' and '3 day' duration papules) ($P > 0{\cdot}05$). The proportions of papules from which bacterial cultures were negative (making no differentiation between high and low microbial populations) were 20% for propionibacteria and 54% for staphylococci and other aerobes.

The geometric mean viable bacterial counts found in colonised pilosebaceous units are also shown in Table 1. The population sizes found in each type of unit were not significantly different (analysis of variance: $P > 0{\cdot}1$ for both propionibacteria and staphylococci). Qualitatively there were no significant differences amongst the distributions of different species and sub-species colonising normal follicles, comedones and inflamed lesions (G test: $P > 0{\cdot}05$).

Methods used for the quantitative determination of *Pityrosporum* were not considered sufficiently reliable to justify numerical analysis; units were considered to be colonised by these yeasts if they were either observed microscopically or cultured. *Pityrosporum* colonisation patterns were similar to those noted for staphylococci and propionibacteria; normal follicles were significantly less frequently colonised than acne lesions (G test: $P < 0{\cdot}001$) but there were no differences amongst the various lesion types (G test: $P > 0{\cdot}05$).

The proportions of papules not colonized by any of the microbial groups were 10% of '1 day' lesions (5 of 50) and 0% '3 day' lesions (0 of 19). These values are close to those predicted by extrapolation from the proportions of lesions colonized by each group, assuming independent assortment (Table 1). This demonstrates that the presence or absence of one microbial group in a lesion has little effect upon the probability of that follicle being colonized by another microbial group.

DISCUSSION

This study has demonstrated that the microbial profile of papules which have been inflamed for a limited period is similar, both quantitatively and qualitatively, to that of non-inflamed comedones. This is perhaps not surprising as inflamed acne lesions are widely believed to be derived from comedones and their sub-clinical precursors ('micro-comedones'). The morphological observations made in this study appear to bear out this assertion, although it is possible that in some of the biopsies from which only apparently normal philosebaceous units were isolated, one of these may have been the cause of the inflammation observed. However, the isolation of papules which were not colonized by micro-organisms is at variance with hypotheses stating that inflammation in acne vulgaris is invariably initiated by microbial activity. Recent investigations into possible roles of micro-organisms in acne have concentrated on *Propionibacterium acnes*. This organism constituted a colonizing population in only 71% of papules and was completely absent in 20% of papules. These findings do not contradict previously published data on the microbiology of papules;[10,11] although the prevalence of staphylococci was lower in the present study than has been reported previously, this is accounted for by the observation of Puhvel and Amirian[12] that comedones extracted from the back were less frequently colonized than were comedones from the face. Shehedah and Kligman[11] concluded that the failure to demonstrate micro-organisms in all inflamed lesions was due to the anti-microbial effect of host immune responses. In the present investigation there was no evidence of appreciable distortion of the viable counts by anti-microbial activity during the development of inflammation. Negative cultures were consistent with microscopic observations and '3 day' duration papules were colonized more frequently than '1 day' duration lesions, although this difference was not statistically significant.

The results presented here suggest that the micro-organisms found in papules are simply an extension of those colonizing comedones and that their presence is not a prerequisite for the development of inflammation in acne. It is probable, however, that when micro-organisms are present in acne lesions they make a significant contribution to the promotion of inflammation. Indeed it would be surprising if they did not, considering the large number of microbial antigens and metabolic by-products which would be released into the dermis from a colonized acne lesion when ruptured. Intradermal injection of *P. acnes* into the ears of rats has also been shown to elicit non-inflammatory changes of possible importance in acne vulgaris most notably the induction of secondary comedones.[6] Micro-organisms may also be the primary trigger of inflammation in some lesions but our results suggest that other components of comedones, such as keratins and lipids, should also be considered as potential inflammatory initiators. It is conceivable that the causes of inflammation vary amongst lesions and individuals, and that this variation could explain apparent paradoxes such as the different responses of individual acne patients to various therapies.

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Immunofluorescent studies in ocular cicatricial pemphigoid

J.N.LEONARD, C.M.HOBDAY, G.P.HAFFENDEN*, C.E.M.GRIFFITHS,
A.V.POWLES, P.WRIGHT† AND L.FRY

Departments of Dermatology and *Histopathology, St. Mary's Hospital, London and the †External Diseases Clinic, Moorfields Eye Hospital, London, U.K.

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SUMMARY

Twenty nine patients with cicatrizing conjunctivitis were studied; 17 with a clinical diagnosis of cicatricial pemphigoid, five with a clinical diagnosis of pseudopemphigoid caused by long-term application of topical medication and seven who had a cicatrizing conjunctivitis from other causes. Biopsies from clinically uninvolved bulbar conjunctiva were taken for direct immunofluorescence and blood was taken for indirect immunofluorescence using normal human conjunctiva, oral mucosa and skin as substrates.

On direct immunofluorescence, *in vivo* bound immunoglobulins were found along the basement membrane in 10 of the 17 patients with cicatricial pemphigoid, one of the five with pseudopemphigoid and two of the seven with a cicatrizing conjunctivitis associated with other diseases. Circulating anti-basement membrane zone antibodies were found only when conjunctiva was used as a substrate. These were present in seven of the patients with cicatricial pemphigoid, three of those with pseudopemphigoid and two of those with a cicatrizing conjunctivitis caused by other diseases.

These results indicate that direct immunofluorescence is a useful, but not absolute diagnostic marker for ocular cicatricial pemphigoid. The results in the pseudopemphigoid group argue that this is an immunologically mediated disorder indistinguishable from spontaneous cicatricial pemphigoid and probably triggered by the drugs. The presence of circulating antibodies should allow for precise identification of the antigen involved in cicatricial pemphigoid using SDS electrophoresis and Western blot analysis.

Cicatricial pemphigoid (CP) is a rare disease affecting the skin and mucous membranes. It is characterized by the development of sub-epidermal blisters which tend to recur in the same site and eventually heal with fibrosis and scarring. When the skin or oral mucous membrane are

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Correspondence: Dr J.N.Leonard, Dermatology Department, St. Mary's Hospital, Praed Street, London W2 1NY, U.K.

involved the diagnosis of the disease is straightforward; it is suggested by the clinical features and confirmed by direct immunofluorescence of perilesional tissue which shows a deposition of immunoglobulins along the basement membrane. With indirect immunofluorescence a circulating anti-basement membrane zone (BMZ) antibody can be demonstrated in a proportion of patients which supports an autoimmune basis for the disease.[1–3]

Blisters are rarely seen on the conjunctiva in ocular CP. Patients usually present with features of a cicatrizing conjunctivitis resulting from sub-conjunctival fibrosis. The earliest changes are loss of normal inner canthal architecture which alone or with quiet fornix shallowing can be completely asymptomatic. More advanced disease may show strikingly asymmetrical or symmetrical changes in the conjunctival fornices, and acute ulcerative lesions occur.[4] If eye involvement occurs in isolation the disease has to be distinguished from a large number of other conditions that result in a cicatrizing conjunctivitis[5] (Table 1). Many of these may be diagnosed on clinical grounds, but in some situations these criteria are unreliable. Previous attempts using direct immunofluorescence on conjunctival biopsies have given rise to inconsistent results, with immunoglobulins being demonstrated in 20% to 67% of patients tested.[6–10] In addition, the highest incidence of positive biopsies came from studies in which the most active area of disease was biopsied. In our experience, biopsy of active conjunctival fornix disease in ocular CP aggravates the scarring and is contraindicated. A significant incidence of circulating anti-BMZ antibody has only been reported in ocular CP in one study, and only when normal conjunctiva was used as a substrate. This suggested that the antigen was tissue specific and not found in other epithelia.[11] The purpose of the present study was to investigate the value of immunofluorescence as a diagnostic test for ocular CP in patients with cicatrizing conjunctivitis, using biopsies

TABLE 1. Causes of cicatrizing conjunctivitis

- Oculocutaneous disorders
  - Cicatricial pemphigoid
  - Linear IgA disease
  - Dermatitis herpetiformis
  - Epidermolysis bullosa
  - Erythema multiforme
  - Toxic epidermal necrolysis
  - Chronic atopic keratoconjunctivitis
  - Rosacea
- Other associated systemic disorders
  - Sjögren's syndrome
  - Inflammatory bowel disease
  - Chronic graft versus host disease
  - Immune complex mediated disease
- Physical
  - Heat
  - Ionizing radiation
  - Chemical burns
- Infection
  - Trachoma
  - Membranous conjunctivitis (viral and bacterial)
- Drug induced
  - Systemic
  - Topical (pseudopemphigoid)

from clinically uninvolved bulbar conjunctiva to avoid aggravating the disease process. In addition, the antigenic specificity of the circulating anti-BMZ antibody was investigated using a range of substrates for indirect immunofluorescence.

METHODS

Twenty-nine patients were studied. Seventeen patients had a clinical diagnosis of ocular CP. Two of these patients had oral ulceration in addition to the eye disease and had previously had an oral mucous membrane biopsy to confirm the diagnosis. In a further two patients the ocular disease was quiescent at the time of biopsy. Five patients had a clinical diagnosis of pseudopemphigoid (all of these patients had been using long term topical therapy for the treatment of glaucoma) and seven patients had a cicatrizing conjunctivitis associated with other diseases. Patient details are given in Table 2.

Biopsy procedure

Biopsies from the bulbar conjunctiva were taken either under local anaesthesia or, if another therapeutic procedure such as lid margin cryotherapy was to be performed, under general anaesthesia. The biopsy was taken from clinically uninvolved bulbar conjunctiva by lifting a small tent of tissue with forceps and undercutting with scissors. No sutures were used. A sterile pad was applied to the eye for 24 h only and chloramphenicol drops were instilled 4 times daily for 5 days. The biopsy sites all healed within 5 days and no scarring or fibrosis ensued. The conjunctival biopsy was embedded in OCT medium (Lab-Tek Inc, Stoke Poges, Slough, U.K.), frozen in liquid nitrogen and stored at −80°C. A 10 ml blood sample was taken from each patient at the time of conjunctival biopsy and the serum separated and stored at −80°C.

Immunofluorescence

The biopsies were examined by direct immunofluorescence using standard methods[12] with FITC-labelled sheep anti-human IgA, IgG, IgM, C_3 and fibrinogen (Dako, High Wycombe, Bucks, U.K.). The presence and class specificities of circulating anti-BMZ antibodies were investigated using standard indirect immunofluorescent techniques.[12] Fresh specimens of

TABLE 2. Details of patients studied

Clinical diagnosis	No. of patients	Sex	Age (years) (mean and range)	Duration of disease (years) (mean and range)
Cicatricial pemphigoid	17	9M, 8F	66 (51–80)	5 (0·3–13)
Pseudopemphigoid	5	2M, 3F	62 (24–78)	5 (4–12)
Other diseases*	7	3M, 4F	41 (27–60)	7 (2–20)

* Ulcerative colitis
Linear IgA disease + ulcerative colitis
Radiotherapy for Bowen's disease of the conjunctiva
Old trachoma infection
Chronic staphylococcal lid infection
Behçet's disease
Keratoconjunctivitis sicca

TABLE 3. Immunofluorescence results from patients with a clinical diagnosis of cicatricial pemphigoid

Patient no.	Sex	Age (years)	Duration of disease (years)	Immunofluorescence Direct (conjunctiva)	Immunofluorescence Indirect (conjunctiva)*
1	F	70	2	IgA, IgG, IgM, C_3, fibrinogen	−ve
2	M	74	7	IgA, IgG, C_3, fibrinogen	−ve
3	F	73	0·3	IgA, IgG, C_3, fibrinogen	IgA, IgG
4	M	51	6	IgA, IgG, C_3	IgA, IgG
5**	F	67	4	IgA, IgG	IgA, IgG, IgM
6	M	75	6	IgG, IgM, fibrinogen	IgG, IgM
7**	M	62	6	IgA, C_3	IgA, IgG
8	M	64	3	IgA, fibrinogen	IgG
9	F	66	1	IgG, fibrinogen	−ve
10	F	53	3	IgA	−ve
11	F	58	15	Fibrinogen	IgA
12	M	70	7	Fibrinogen	−ve
13	F	80	10	Fibrinogen	−ve
14***	M	73	5	Fibrinogen	−ve
15***	M	58	6	Fibrinogen	−ve
16	M	62	2	−ve	−ve
17	F	58	8	−ve	−ve

* All patients were negative when oral mucous membrane and skin were used as substrates.

** These patients had oral mucous membrane lesions

*** These patients were in clinical remission at the time of biopsy.

normal human skin, oral mucous membrane and conjunctiva were used as substrates. Sera were scored as positive if the titre exceeded 1:20.

RESULTS

Cicatricial pemphigoid

The results are summarized in Table 3. On direct immunofluorescence 10 patients had immunoglobulins deposited along the basement membrane of the conjunctiva. In eight patients IgA was deposited (Fig. 1), in seven IgG and in two IgM. One patient had all three classes of immunoglobulin, four had both IgA and IgG, one had IgG and IgM and four had either IgA or IgG. There was no relationship between class of immunoglobulin and specific clinical features. Five patients had deposition of C_3 along the basement membrane and 11 had fibrinogen.

On indirect immunofluorescence, circulating anti-BMZ antibodies were found in seven patients. Five patients had IgA, six had IgG (Fig. 2) and two had IgM class antibodies when normal human conjunctiva was used as a substrate. None of the sera was positive when normal human skin or oral mucous membrane was used as a substrate.

There was good correlation between the direct and indirect immunofluorescence results in five of the seven patients found to have circulating anti-BMZ antibodies (Patients 3–7). In the

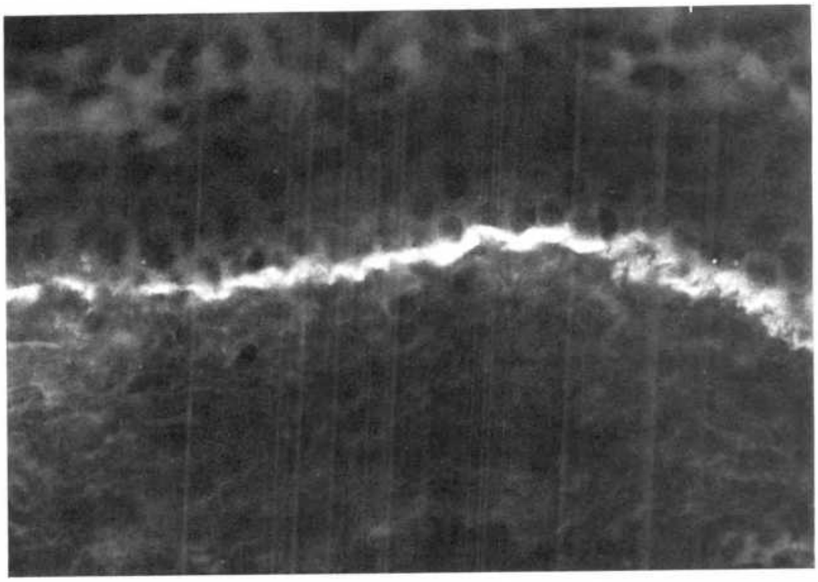

FIGURE 1. Direct immunofluorescence of a positive conjunctival biopsy from a patient with ocular cicatricial pemphigoid, showing a homogeneous linear pattern of IgA deposition along the basement membrane.

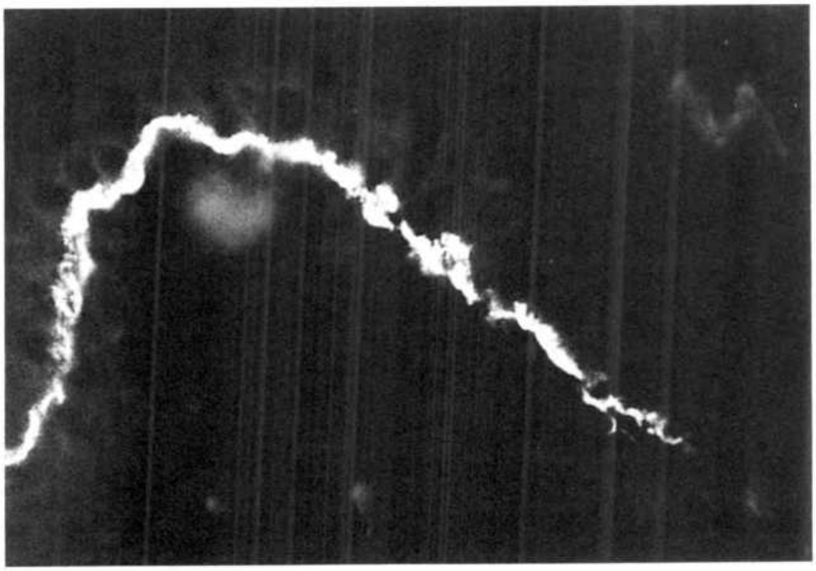

FIGURE 2. Indirect immunofluorescence using normal human conjunctiva as substrate, demonstrating the presence of a circulating anti-basement membrane zone antibody of IgG class in the serum of a patient with ocular cicatricial pemphigoid.

TABLE 4. Immunofluorescence results from patients with a clinical diagnosis of pseudopemphigoid

Patient no.	Sex	Age (years)	Duration of disease (years)	Immunofluorescence	
				Direct (conjunctiva)	Indirect (conjunctiva)*
18	M	68	4	IgG, fibrinogen	IgA, IgG
19	F	58	5	IgM (granular)	IgA, IgG
20	M	79	4	Fibrinogen	IgA, IgG
21	F	64	12	−ve	−ve
22	F	24	2	−ve	−ve

* All patients were negative when oral mucous membrane and skin were used as substrates.

TABLE 5. Immunofluorescence results from patients with cicatrizing conjunctivitis related to other diseases

Patient no.	Associated diseases	Sex	Age (years)	Duration of disease (years)	Immunofluorescence	
					Direct (conjunctiva)	Indirect (conjunctiva)*
23	Ulcerative colitis	F	34	2	IgA, IgG, IgM, fibrinogen	IgG
24	Linear IgA disease + UC	F	39	4	IgA, fibrinogen	−ve
25	Radiotherapy	F	46	3	Fibrinogen	IgG
26	Staphylococcal infection	F	60	3	Fibrinogen	−ve
27	Trachoma	M	53	10	Fibrinogen	−ve
28	Behçet's disease	M	28	2	−ve	−ve
29	Keratoconjunctivitis sicca	M	27	20	−ve	−ve

* All patients were negative when oral mucous membrane and skin were used as substrates.

two patients who had previously had an oral mucous membrane biopsy (Patients 5 and 7), one had IgG and the other IgA and IgG deposited along the basement membrane of perilesional oral mucosa. Neither of these two patients had circulating anti-BMZ antibodies using normal human oral mucosa as a substrate. Two patients were in clinical remission at the time of biopsy (Patients 14 and 15) and neither had *in vivo* bound immunoglobulin deposition or circulating anti-BMZ antibodies.

Pseudopemphigoid

The results are summarized in Table 4. Only one patient had a linear deposit of immunoglobulin along the basement membrane zone of the conjunctiva (Patient 18). One other patient (Patient 19) had granular deposits of IgM. Two patients had linear deposition of fibrinogen along the basement membrane. Three patients had circulating anti-BMZ antibodies using normal human conjunctiva as substrate. In each instance the circulating antibodies were of IgA and IgG class. None of the sera was positive when normal human skin or oral mucous membrane was used as a substrate.

Cicatrizing conjunctivitis associated with other diseases

The results are summarized in Table 5. Two patients were positive on direct immunofluorescence for immunoglobulins. Patient 23 (with ulcerative colitis) had IgA, IgG, and IgM deposits along the basement membrane. Patient 24 (with linear IgA disease and ulcerative colitis) had *in vivo* bound IgA. Four patients had basement membrane deposition of fibrinogen. Two patients had circulating anti-BMZ antibodies detected when normal human conjunctiva was used as a substrate—Patient 23 with ulcerative colitis and Patient 25 who had a cicatrizing conjunctivitis associated with previous radiotherapy. None of the sera was positive when normal human oral mucous membrane or skin was used as substrate.

DISCUSSION

This study has confirmed that patients with ocular CP have deposits of immunoglobulins along the basement membrane zone. Ten of the 17 patients (58%) with a clinical diagnosis of ocular CP had a positive biopsy. This incidence compares favourably with other studies which ranged from 20% to 67%.[6–10] The highest incidence of positive results was reported by Mondino *et al.*[10] who studied 12 biopsies from areas of obvious conjunctival shrinkage in patients with a clinical diagnosis of ocular CP. However, only two of 10 biopsies from clinically uninvolved conjunctiva were positive.

Seven patients in the present study with a clinical diagnosis of ocular CP did not have *in vivo* bound immunoglobulins demonstrated by direct immunofluorescence. In two of these the disease was in clinical remission at the time of the study and this may explain the negative result. It is not clear why the remaining five patients were negative. It is possible that the disease had become inactive or that deposition of immunoglobulin was not uniform.

One of the 5 patients with a clinical diagnosis of pseudopemphigoid had a linear deposition of immunoglobulin on conjunctival biopsy. There has been one previous report of immunoglobulin deposition in a patient with pseudopemphigoid.[13] Pseudopemphigoid is clinically indistinguishable from ocular CP. It is a rare complication of long-term usage of topical therapy especially antiglaucoma medication such as guanethidine or adrenalin drops. The mechanism by which these drugs produce a cicatrizing conjunctivitis is unknown. It is possible that they have an effect on the local immune system in the eye leading to increased activity of fibroblasts. Alternatively, the drugs may render certain components of the basement membrane zone immunogenic as a result of long term toxic damage, giving rise to an autoimmune disease. A further possibility is that patients who develop pseudopemphigoid have a predisposition to develop CP and the drug simply acts as a trigger. The phenomenon of pseudopemphigoid may be compared with the situation in which penicillamine and captopril have been shown to cause pemphigus which is indistinguishable clinically, histologically, immunologically and therapeutically from the spontaneously occurring disease.[14,15] Drug induced pemphigus does not necessarily go into remission when the drug is withdrawn. The findings in the present study suggest that pseudopemphigoid is an immunologically mediated disease. One of the patients with pseudopemphigoid had granular deposition of IgM. This was an unexpected finding. Granular deposits of IgA have been described previously in ocular cicatricial pemphigoid and have been attributed to the deposition of immune complexes.[16]

In the patients who had a cicatrizing conjunctivitis associated with other diseases only two had *in vivo* bound immunoglobulins. The patient with linear IgA disease would have been expected to have *in vivo* bound IgA as these deposits are a diagnostic feature of the cutaneous disease.[17] The findings in the patient with ulcerative colitis may be the result of two independent diseases—ulcerative colitis and ocular CP—or it may be that the cicatrizing conjunctivitis had arisen as a consequence of the bowel disease.

C_3 deposits were found in only five patients in the present study, all with CP. The pathogenetic significance of this finding is not clear. Fibrinogen was found in the conjunctiva along the basement membrane in a high proportion of patients. It was found equally in all three disease groups and is probably a reflection of the inflammation that occurs in the tissues. Fibrinogen is found in other bullous skin diseases and no primary pathogenetic significance has been attributed to its presence.

Circulating anti-BMZ antibodies were found in seven of the 17 CP patients, three of the five patients with pseudopemphigoid and two of the seven patients with other diseases. They were only demonstrated when fresh human conjunctiva was used as the substrate, indicating that conjunctival basement membrane contains antigens that are not found in oral mucosa or skin. There has only been one previous well documented report of circulating anti-BMZ antibodies in ocular cicatricial pemphigoid.[11] These investigators found antibodies against conjunctival basement membrane in six of 12 patients with ocular CP using fresh human conjunctiva as substrate. Of 16 patients with other corneal or conjunctival disease, they reported weak basement membrane zone staining (IgG class) in one patient with corneal vascularization of unknown cause and one patient with Sjögren's syndrome. Mondino *et al.*,[10] by contrast, found no circulating antibodies in 12 patients studied using normal human conjunctiva and cornea, taken within 2 h of death, as substrate. They also had negative results using normal monkey oesophagus, normal guinea pig oesophagus and guinea pig lip. Bean *et al.*[7] studied serum from 10 patients using 9 different substrates including human conjunctiva but found only three positive results—only one with human conjunctiva. The specificity of the substrate was also reported by Laskaris *et al.*[18] in patients with oral mucous membrane pemphigoid. The results of these studies are in contrast to those of Powell *et al.*[19] who reported a failure of specific human tissue substrates to increase the sensitivity of indirect immunofluorescence testing in cicatricial pemphigoid.

Not all basement membranes are identical antigenically. Previous studies have demonstrated differences between various human epithelia and it is possible that the underlying basement membrane, which is partly synthesized by the epithelial cells, reflects these differences.[20] The finding of a circulating anti-BMZ antibody is important as it should allow identification of the relevant basement membrane antigen by SDS electrophoresis and Western blot analysis.[21] This may help further understanding of the mechanisms that lead to scarring in some bullous diseases, but not in others.

ACKNOWLEDGMENTS

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Effect of PUVA on intestinal calcium absorption

K.E.VIRVIDAKIS, J.D.BROKALAKIS, P.N.SINGHELLAKIS AND T.D.MOUNTOKALAKIS

Second Medical Department and Department of Dermatology University of Athens, Athens, Greece

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SUMMARY

Intestinal calcium absorption increased significantly following 14 days of treatment with photochemotherapy (PUVA) in seven patients with chronic psoriasis. Irradiation with UVA alone over a similar period did not result in significant changes in calcium absorption in an additional seven prosiatic patients. These findings suggest that the increase in serum 25-OH vitamin D levels previously reported in patients treated by PUVA may have important biological consequences.

An increase in the serum concentration of 25-OH vitamin D has been reported in patients with psoriasis treated by photochemotherapy with 8-methoxypsoralen (8-MOP) and long-wave ultraviolet irradiation (PUVA).[1,2] Because vitamin D must be converted to a metabolically active form before it can function, the biological significance of this finding is unclear. One of the major sites of action of the active metabolite 1,25-$(OH)_2$ vitamin D is at the intestinal brush borders, where calcium entry is stimulated.[3] Intestinal calcium absorption, therefore, can be used as a measure of the functional activity of vitamin D. In the present study we have measured intestinal calcium absorption in psoriatic patients before and after PUVA therapy.

METHODS

We studied 14 male patients with chronic plaque psoriasis after obtaining their informed consent. Patients were divided into two groups: Group 1 included seven patients, aged 46–75 years, who were treated with oral 8-MOP, 0·6 mg/kg, followed by whole-body exposure to long-wave (320–400 mm) ultraviolet radiation (UVA), 2h later. Treatment was repeated four times a week with radiation doses starting at 2 J/m^2 and increasing by 1 J/m^2 every second session. The total dose of UVA in each patient over 14 days was 28 J/m^2. Group 2 included seven patients, aged 28–65 years, who were treated with UVA alone without previous administration of 8-MOP. UVA treatment was exactly the same as in Group 1.

Correspondence: Kyriakos E. Virvidakis, M.D. General Hospital of Chania, Chania 73100, Greece.

In each patient, intestinal calcium absorption was measured, before and 14 days after starting treatment, by a double radioisotope technique, using ^{47}Ca as the oral tracer and ^{45}Ca as the intravenous tracer.[4] On the morning of the test the patients, who had been fasting since the previous night, were given a strictly standardized breakfast of crackers and cheese, containing 80 mg calcium. The oral tracer dose, consisting of 25 μCi of ^{47}Ca in 140 mg carrier calcium (as chloride), was given during breakfast and the patients then fasted again for a further 4 h. The intravenous tracer, consisting of 7 μCi ^{45}Ca, was given exactly 2 h after the oral dose. Plasma samples were obtained 24 h later and the ^{47}Ca concentration measured, together with the appropriate standards using a well-type scintillation counter. Plasma ^{45}Ca was measured at least 1 month later, so that the ^{47}Ca content had mostly decayed. Appropriate corrections were made for any residual radioactivity due to the oral tracer by means of the appropriate standards.

The true intestinal calcium absorption coefficient (*a* value) was calculated according to the formula:

$$a = \frac{\%\ \text{oral dose/l plasma}}{\%\ \text{intravenous dose/l plasma}} \times 100$$

This is based on the assumption that the two tracers enter the exchangeable calcium pool simultaneously, so that the ratio of oral and intravenous dose concentration is determined by intestinal absorption only, since all other factors affect both concentrations to the same extent.[4]

Statistical analysis of the results was carried out using Student's paired *t*-test.

RESULTS

The results are shown in Figure 1. Patients treated with PUVA for 14 days showed a significant increase in mean *a* value ($\pm$ SD) from 23·8 ± 1·94 to 38·5 ± 3·86, ($P < 0{\cdot}01$) while no significant

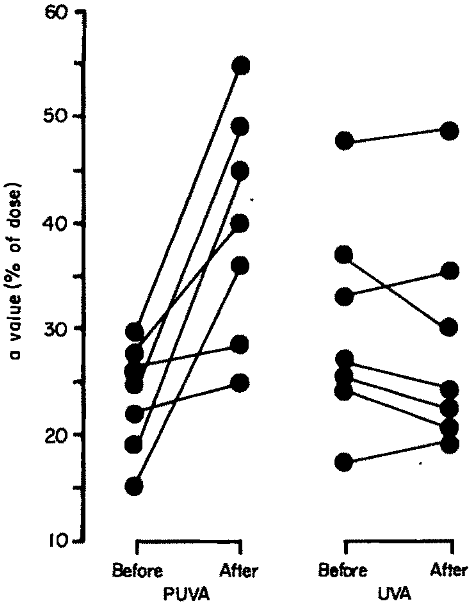

FIGURE 1. Intestinal calcium absorption (*a* value) before and after treatment of psoriatic patients with 8-MOP and UVA (PUVA) or UVA irradiation alone.

change in mean *a* value occurred in the patients exposed to UVA radiation alone (29·0 ± 3·22 and 27·0 ± 3·75, respectively).

DISCUSSION

The combination of oral psoralens with local exposure of the skin to ultraviolet radiation is a highly effective therapy in the control of severe psoriasis. Although immediate side effects are quite tolerable, the possibility of long-term sequelae cannot be ruled out.[5] Some years ago, Rogers *et al.*[1] and Shuster *et al.*[2] reported an increase in serum vitamin D after photochemotherapy with 8-MOP and UVA. Yet, the consequences of these high circulating levels of 25-OH vitamin D were not elucidated by these authors. The best documented effect of vitamin D is to enhance the intestinal transport of calcium.[3] The present finding of a significant increase in intestinal calcium absorption following 2 weeks of treatment with PUVA suggests that the increase in serum 25-OH vitamin D levels in patients treated by PUVA may have important biological consequences.

Mean pre-treatment values of calcium absorption did not differ significantly between the two patient groups. However, three of the patients treated with UVA alone had relatively high pre-treatment values. The lack of change in these patients suggests that the rise in calcium absorption following treatment could be related to the effect of adventitious UVB radiation in the patients with lower basal calcium absorption values indicative of somewhat deficient circulating 1,25-$(OH)_2$ vitamin D levels. However, a difference in response to the two treatments was evident even between patients with comparable calcium absorption values, while any adventitious UVB radiation would be expected to affect both groups similarly.

Although it is difficult to speculate on the possble mechanism by which PUVA affects the production of 25-OH vitamin D, the fact that both the increase in serum 25-OH vitamin D levels reported by Rogers *et al.*[1] and Shuster *et al.*[2] and the enhancement of intestinal calcium absorption observed in the present study were not seen with UVA alone (or with 8-MOP alone, in the case of 25-OH vitamin D levels[2]) suggests that such a mechanism may be linked to the photochemical reaction that results from the exposure of psoralens to UVA. This reaction seems to be related, at least in part, to the binding of psoralens to DNA by the action of UVA.[5]

The known potential hazards of excessive vitamin D are hypercalcaemia and hypercalciuria. In severe, unrecognized, or untreated vitamin D intoxication a widespread ectopic calcification results, especially in the kidneys, causing renal failure.[6] Most psoriatic patients clear in an average of 20 treatments with PUVA. After clearing, however, the patients usually need maintenance exposure once per week or less frequently. Monitoring of serum and urinary calcium may, therefore, be advisable in these patients.

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Clinical features and course of type A and type B vitiligo

M.KOGA AND T.TANGO*

Department of Dermatology, Tokyo Medical College Hospital and * Division of Clinical Epidemiology, The Tokyo Metropolitan Institute of Medical Science, Tokyo, Japan

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SUMMARY

Four hundred and eighty one cases of vitiligo (acquired idiopathic depigmentation) were studied clinically. They were divided into two types, type B, in which depigmented patches are confined to a definite dermatome in the same manner as herpes zoster, and type A, which included all cases of vitiligo not classified as type B. The ratio of type A:type B was approximately 3:1. Onset of type A vitiligo could occur at any age, whereas type B generally affected the young. The activity of type B vitiligo usually ceased after one year, following rapid spread over the particular dermatomal area. However, in type A vitiligo new depigmented patches continued to appear throughout the patients' lives. Association with halo naevus and Kōbner's phenomenon were observed exclusively in type A. Association with diseases with a proven or suggested allergic or immunological aetiology was more often found in type A. These findings support our hypothesis that type A and type B vitiligo have a different pathogenesis and that autoimmune mechanisms play a role only in type A.

In the past, hypotheses concerning the pathogenesis of vitiligo have been confusing and contradictory. However, autoimmune mechanisms have been considered important by many authors.[1–3] We have suggested that vitiligo can be divided into two types: type A, caused by autoimmune mechanisms and type B which results from the dysfunction of sympathetic nerves in the affected area.[4] If this is the case, the clinical course and features of the two types should be different, and this has been investigated in the present study.

METHODS

Patients

Four hundred and eighty one patients with vitiligo who were seen in our clinic over the 10-year

Correspondence: Dr M.Koga, Department of Dermatology, Tokyo Medical College Hospital, 6-7-1 Nishishinjuku, Shinjuku-ku, Tokyo, Japan.

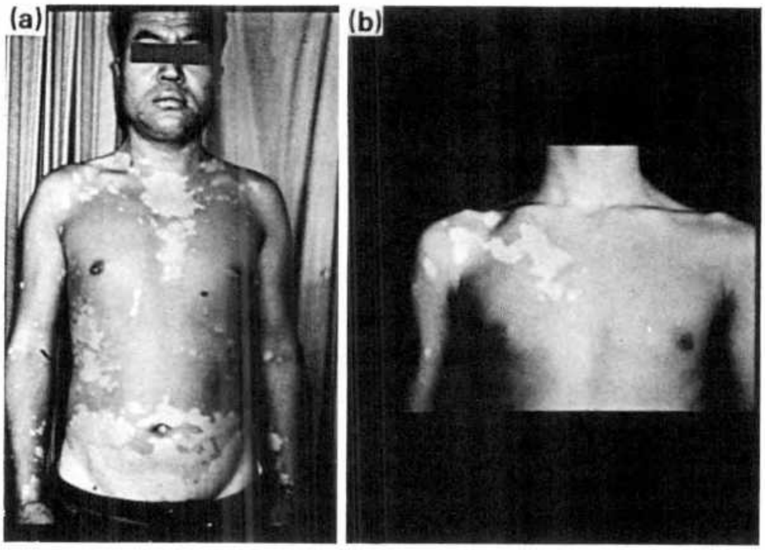

FIGURE 1. Clinical features of (a) type A vitiligo and (b) type B vitiligo.

period 1971–1980 were studied. Thirty six patients with halo naevus, some of whom had associated vitiligo, were also studied.

Clinical data

When patients were seen for the first time a detailed history was taken, including age at onset of vitiligo, progression of the vitiligo and any associated diseases in the patients and their close relatives. The distribution pattern of the patches of depigmentation was examined and the patients were divided into two groups; type B, where depigmented patches were confined to a definite dermatome in the same manner as herpes zoster, and type A, which included all cases of vitiligo not classified as type B. The depigmented patches in type A vitiligo were distributed irregularly, although they tended towards a symmetrical distribution during the progression of the disease (Fig. 1).

All patients were examined for halo naevi or occurrence of Köbner's phenomenon. In the course of treatment over many years the appearance of new depigmented patches was carefully recorded.

Statistical analysis

The results were analysed using the Wilcoxon rank sum test, Fisher's exact test and the χ^2 test.

RESULTS

Incidence of type A and type B vitiligo

Out of 481 cases of vitiligo 350 were type A, and 134 were type B; an approximate ratio of type A:type B of 3:1. There were three cases who when first seen had type B vitiligo and who then developed generalized vitiligo over the whole body, which was considered to be the development of type A vitiligo from type B. There was a slight preponderance of females among

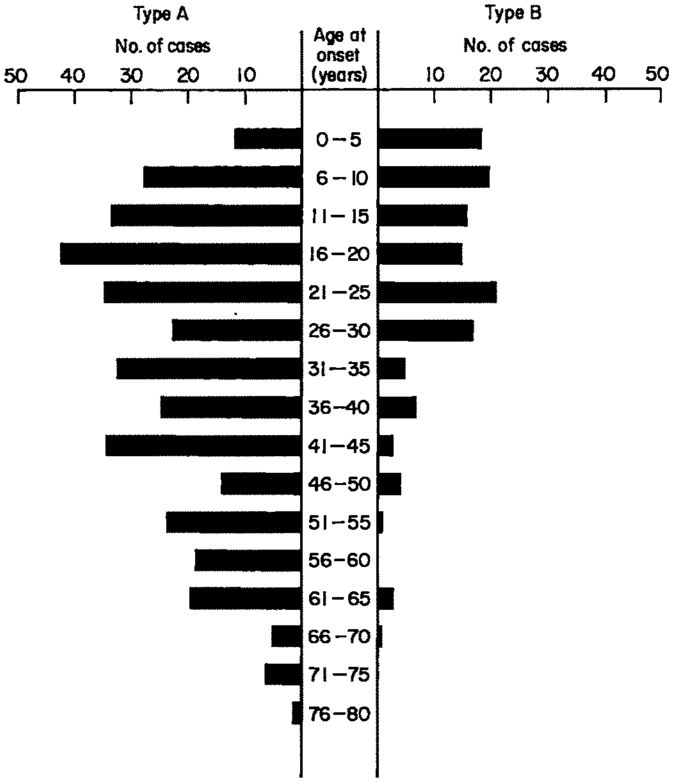

FIGURE 2. Age at onset of type A and type B vitiligo.

cases of both types, probably because vitiligo causes more cosmetic and psychological problems in females than in males.

Age at onset

The onset of type A vitiligo occurred over a wide age range, while most patients with type B became affected while they were young (Fig. 2). Of the 134 cases of type B vitiligo, 110 (82%) noticed the first depigmented patches before the age of 30, whereas only 169 of the 350 cases of type A vitiligo (48%) were suffering from the disease by that age. There was a statistically significant difference between age distributions in the two types ($P < 0{\cdot}001$; χ^2 test).

Disease progression

Disease progression was assessed in 94 cases of type A and 26 cases of type B on the basis of a reliable past history and direct observation by the author.

As shown in Figure 3, the progression of type B vitiligo occurred over a relatively short period. The disease tended to progress rapidly immediately after onset, but the activity often ceased after 1 year. With type A vitiligo, new depigmented patches appeared continuously for 20 years or more after onset, and presumably this would continue for the rest of the patient's life.

The median period of progression of vitiligo was 0·7 years in type B, which was significantly shorter than that in type A (5·1 years) ($P < 0{\cdot}001$, Wilcoxon rank sum test). In 17 of the 26 cases

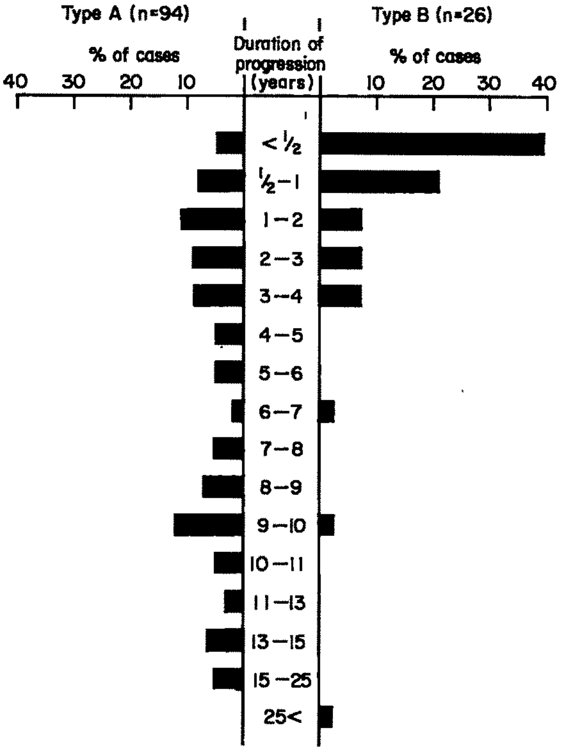

FIGURE 3. Duration of disease progression in type A and type B vitiligo.

with type B vitiligo (65%) disease activity ceased within 1 year, compared with 11 of the 94 patients with type A (12%) ($P < 0{\cdot}001$, χ^2 test).

Association with halo naevus

Out of 36 cases of halo naevus, 23 were associated with, or developed vitiligo, which was type A in all cases. No case of halo-naevus was associated with type B vitiligo. Thus there was a statistically significant association with type A ($P < 0{\cdot}001$, Fisher's exact test).

Association with Köbner's phenomenon

Köbner's phenomenon, the appearance of linear or macular depigmented lesions on the arms, elbows and knees after scratches or trauma, was found in 18 cases of vitiligo all of which were type A. Thus, Köbner's phenomenon is significantly associated with type A vitiligo ($P < 0{\cdot}001$; Fisher's exact test).

Association with diseases with a proven or suggested allergic or immunological aetiology

As shown in Table 1, diseases with a proven or suggested allergic or immunological aetiology, such as urticaria, asthma and diabetes, and including tuberculosis, as this can affect the immune system,[5] were found more frequently in the personal and family histories of patients with type A vitiligo. Although not age-matched, 95 of the 350 patients with type A vitiligo (27%) had a personal or family history of an allergic or immunological disease which was a significantly

TABLE 1. History of diseases with a proven or suspected allergic or immunological aetiology in patients with type A or type B vitiligo and their close relatives

	No. of patients	
	Type A ($n = 350$)	Type B ($n = 134$)
Vitiligo (in a relative)	23	6
Diabetes	17	2
Tuberculosis	12	0
Urticaria	10	0
Asthma	8	0
Alopecia areata	6	0
Drug eruption	6	3
Thyroid disease	3	0
Contact dermatitis	3	0
Joint pain	3	0
Pernicious anaemia	2	0
Pustulosis palmaris et plantaris	2	1
Total	95	14

higher frequency than that in type B, where only 12 of the 134 patients (9%) had a personal or family history of such disease ($P < 0{\cdot}001$, χ^2 test).

DISCUSSION

The results of the present study showed that the two types of vitiligo differ significantly in their characteristic features. Onset is predominantly at an early age in type B, while type A can occur at any age. Once it occurs, type B vitiligo spreads rapidly over the affected dermatomal area and then the activity usually ceases after a short period. The depigmented patches then persist unchanged for the rest of the patient's life, except in some cases in which type B vitiligo developed in childhood and partially disappeared spontaneously during puberty. A very different behaviour is seen in type A vitiligo, which progresses continuously with alternate periods of remission and exacerbation. New depigmented patches continue to appear 10 or 20 years after onset and can affect the skin of the whole body in the later stages. The pattern of progression is similar to that in other autoimmune diseases, for example SLE. Three of 134 cases of type B vitiligo developed into type A, suggesting that type B vitiligo can trigger an autoimmune process in the melanogenic system.

Halo naevus is suspected to have an autoimmune pathogenesis[6,7] and has a high rate of association with vitiligo.[8] In the present study the vitiligo associated with halo-naevus was exclusively type A. Köbner's phenomenon with its probable connection with systemic illness was also associated with type A vitiligo exclusively. The association with other diseases with a proven or suggested allergic or immunological aetiology was frequent in type A and much less frequent in type B.

These findings strongly suggest that the two types of vitiligo have a different pathogenesis and that autoimmune mechanisms are restricted to type A vitiligo.

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Active participation of eosinophils in patch test reactions to inhalant allergens in patients with atopic dermatitis

C.A.F.M.BRUYNZEEL-KOOMEN, D.F. VAN WICHEN, C.J.F.SPRY*, P.VENGE† AND P.L.B.BRUYNZEEL‡

Departments of Dermatology and ‡ Pulmonary Disease, University Hospital, Utrecht, The Netherlands, * Department of Immunology, St. George's Hospital Medical School, London, U.K. and † Department of Clinical Chemistry, University Hospital Uppsala, Sweden

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SUMMARY

Intracutaneous testing and patch tests with house dust mite and grass pollen allergens were performed in patients with atopic dermatitis. Only patients with an immediate type skin reaction to house dust mite or grass pollen allergens showed a positive patch test reaction to these allergens 24–48 h after testing. Occasionally positive patch test reactions at 20 min, 2 h and 6 h were also observed. Patch test reactions were not found in normal controls or atopic patients without atopic dermatitis. Analysis of the cellular infiltrate demonstrated an influx of eosinophils into the dermis, starting from 2–6 h after patch testing. Immunostaining with antibodies against granular constituents of the eosinophils revealed that the infiltrating eosinophils were in an activated state and had lost part of their granular contents. At 24 h eosinophils also appeared in the epidermis.

Electron microscopy showed that in the epidermis, some eosinophils were in close contact with Langerhans cells, suggesting a cell–cell interaction. Taken together, these results strongly suggest an active role for eosinophils in patch test reactions to inhalant allergens in atopic dermatitis patients.

Elevated serum IgE levels with a specificity for a wide variety of inhalant allergens may be observed in 90% of patients with atopic dermatitis (AD).[1] Moreover, these patients show immediate type skin test reactions and positive patch test reactions to inhalant allergens. These patch test reactions, which are delayed,[2–4] are specific to patients with AD. The first report on these patch test reactions described the presence of basophils in the dermal infiltrate 48 h after testing.[2] A cutaneous basophil hypersensitivity was thought to underlie this reaction pattern.

Correspondence: Dr C.A.F.M.Bruynzeel-Koomen, Department of Dermatology, University Hospital, Catharijnesingel 101, 3511 Gv Utrecht, The Netherlands

Unfortunately, this finding could not be confirmed by others.[3,4] Reitamo *et al.*[3] reported the presence of OKT6+ cells (Langerhans' cells (LC) or indeterminate cells) and Ia+ T lymphocytes in the dermis 48 h after patch testing.

Since analysis of the infiltrate in clinically involved skin from AD patients suggests the involvement of a cell mediated immune response,[5,6] it has been suggested that LC play a role in the reaction mechanism underlying these patch test reactions.[7] Epidermal LC from patients with AD bear IgE molecules,[8] and therefore epicutaneously applied inhalant allergens may bind to allergen specific IgE molecules on these LC and, since LC have a potent antigen presenting capacity,[9] induce a delayed hypersensitivity reaction. This is supported by the *in vitro* finding that inhalant allergens can induce a T lymphocyte proliferative response in atopic patients.[10]

Furthermore, patients with AD frequently show elevated blood eosinophil counts, and eosinophil derived major basic protein has been demonstrated in eczematous skin from AD patients.[11] This suggests that eosinophils may also play a role in the pathogenesis of AD. Evidence is accumulating that eosinophils actively participate in late phase allergic reactions in different tissues.[12–14] In addition to an influx of basophils[2] and activated T lymphocytes,[3] the above studies also included reports of an influx of eosinophils into the dermis 24–48 h after patch testing, which suggests that eosinophils may also play a role in these patch test reactions.

In the present study we have investigated the role of eosinophils in patch test reactions to inhalant allergens in patients with AD.

METHODS

Subjects

Fifteen patients with AD, 10 atopic patients with asthma or rhinitis or both without AD, and 10 normal non-atopic subjects, aged 18–38 years, were studied after giving their informed consent. The diagnosis of AD was made according to the criteria of Hanifin *et al.*[15] The patients with AD were tested during clinical remission. In the patients with AD the number of eosinophils in the peripheral blood was $0{\cdot}2-0{\cdot}4\times10^9$/l.

Skin tests

Patients and controls were tested intracutaneously with 0·05 ml of a commercial house dust mite (HM) allergen preparation (*Dermatophagoides pteronyssinus*) and a mixed grass pollen (GP) allergen preparation (*Lolium perenne, Dactylis glomerata* and *Festuca rubra*) (Haarlems Allergenen Laboratorium, Haarlem, The Netherlands).

Epicutaneous patch tests were performed with Leucotest (Beiersdorf AG, Hamburg, F.R.G.), on clinically normal skin on the back. Before application of the patch tests, most of the stratum corneum was stripped off by 15 applications of adhesive tape, leaving only a thin residual layer of stratum corneum. The concentration of the aqueous allergen preparations used for patch testing was 100 times the concentration used for intracutaneous testing. The diluents alone were used as negative controls. Intracutaneous test reactions were read after 20 min, 6 h and 24 h. All patch test reactions were read after 20 min, and 2, 6, 24 and 48 h. Erythema was recorded as +, erythema with induration or papules or both as ++.

Skin biopsies

Skin biopsies were taken from positive patch test reaction sites. One biopsy was fixed in Karnovsky's solution. One part of this biopsy was embedded in plastic (glycolmethacrylate) and 2 μm sections were stained with haematoxylin and eosin for identification of eosinophils, and a modified Giemsa's stain[16] for mast cells and basophils. The other part was prepared for electron microscopy. A second biopsy was snap frozen in liquid nitrogen for immunostaining of cell surface markers. The following antibodies were used: OKT6 (Ortho Diagnostic Systems Inc., Raritan, NJ, U.S.A.) Leu 1, Leu 2a, Leu 3a (Becton Dickinson, Ca, U.S.A.) and anti-human IgE (Central Laboratory, Blood Transfusion Service, Amsterdam, The Netherlands). Immunostaining was performed using an indirect immunoperoxidase method.[8] A polyclonal rabbit anti-human eosinophil cationic protein antibody (R-a-ECP) was used at a dilution of 1:400. This antibody recognizes the storage and secreted forms of human eosinophil cationic protein (ECP). A monoclonal mouse anti-ECP antibody (EG2) was used, which is specific for ECP from activated eosinophils and also binds to the secreted forms of ECP and eosinophil protein-X (EPX) (identical to eosinophil derived neurotoxin).[17] Activated (EG2+) eosinophils have been demonstrated in skin biopsies from urticaria patients.[18] EG2 antibody was used at a dilution of 1:10. As secondary antibodies, alkaline phosphatase conjugated goat anti-rabbit IgG or goat anti-mouse IgG were used. The staining reaction was developed with naphtol AS-MX phosphate and fast red TR with 1mM levamisole to inhibit endogeneous alkaline phosphatase. The numbers of inflammatory cells in the dermis, stained with haematoxylin and eosin, Giemsa or specific antibodies, were counted at × 400 magnification. At least 300 cells were counted per skin section. Double immuno-staining of frozen skin sections was performed by co-incubation of OKT6 with R-a-ECP antibodies and of monoclonal anti-human IgE with R-a-ECP antibodies. As secondary antibodies, goat anti-mouse antibody (peroxidase conjugated) and swine anti-rabbit antibody (alkaline phosphatase conjugated), respectively, were used. The peroxidase reaction was developed at pH 4·8–5·0. Controls were performed by substitution of the primary antibodies by non-immune mouse serum.

Serum IgE

The total serum IgE level was measured using the paper-radioimmunosorbent test (PRIST) and allergen specific serum IgE was measured using the radio-allergosorbent test (RAST, Pharmacia, Uppsala, Sweden). Total serum IgE values were expressed in kU/l. Specific serum IgE values (RAST) were expressed as a percentage of the total amount of added labelled anti-human-IgE which bound to the allergen-coated disc. These values were corrected for the blank binding of labelled anti-human-IgE to the disc.

RESULTS

Skin tests

The results of intracutaneous testing with HM and GP allergens are shown in Table 1, together with the total and allergen specific serum IgE levels. All 15 patients with AD showed an immediate reaction to HM allergen. Reactions were not observed at 6 and 24 h. Immediate type skin reactions to GP allergen were present in 13 of the 15 patients. Table 2 shows the results of patch tests with HM and GP allergens. Twelve of the 15 AD patients showed a positive patch test reaction to HM allergen with a maximum at 24–48 h after testing. Patch test reactions at 24–48 h showed erythema, or erythema with induration or papules. Patch test reactions to HM allergen were seen also at 20 min and 2 and 6 h and showed erythema or erythema and induration. The number of positive patch test reactions to GP allergen was smaller than to HM allergen; only four out of 13 AD patients with an immediate type skin reaction to GP allergen showed a positive patch test reaction to GP allergen with a maximum at 24–48 h after testing. Patient 12 showed a patch test reaction to GP with a maximum at 6 h. Patch tests with control

TABLE 1. The results of intracutaneous testing of house dust mite (HM) and grass pollen (GP) allergens, and total serum IgE (PRIST) and allergen specific serum IgE (RAST) levels in 15 patients with atopic dermatitis

Patient no.	Allergen	Tests read after 20 min	6 h	24 h	PRIST kU/l	RAST % binding
1	HM	+	—	—	3800	28
	GP	+ +	—	—		29
2	HM	+	—	—	2300	24
	GP	—	—	—		1
3	HM	+	—	—	1900	33
	GP	+	—	—		22
4	HM	+	—	—	15000	42
	GP	+ +	—	—		36
5	HM	+ +	—	—	1200	26
	GP	—	—	—		1
6	HM	+	—	—	110	3
	GP	+ +	—	—		1
7	HM	+	—	—	3500	2
	GP	+	—	—		4
8	HM	+	—	—	920	34
	GP	+	—	—		20
9	HM	+	—	—	nd	nd
	GP	+	—	—		
10	HM	+	—	—	3600	14
	GP	+ +	—	—		33
11	HM	+	—	—	4000	26
	GP	+ +	—	—		30
12	HM	+	—	—	21000	33
	GP	+	—	—		32
13	HM	+ +	—	—	4500	32
	GP	+ +	—	—		30
14	HM	+	—	—	17000	17
	GP	+	—	—		36
15	HM	+	—	—	9000	15
	GP	+ +	—	—		23

+ = weal diameter 7–10 mm
+ + = weal diameter > 10 mm
— = no reaction
nd = not done

diluents were negative in all patients. None of the atopics without AD and none of the normal non-atopic controls showed patch test reactions to HM or GP allergens 24–48 h after testing.

Haematoxylin and eosin and Giemsa's staining of biopsies

In the skin biopsies from positive patch test reactions to HM and GP, epidermal spongiosis and vesiculation was present at 24–48 h after testing. The results of haematoxylin and eosin staining for eosinophils in the biopsies are given in Table 3. The maximal eosinophil infiltration in

TABLE 2. The results of patch tests with house dust mite (HM) and grass pollen (GP) allergens in 15 patients with atopic dermatitis

Patient no.	Allergen	Tests read after				
		20 min	2 h	6 h	24 h	48 h
1	HM	++	+	—	++	++
	GP	++*	—	+	++*	++*
2	HM	++	+	—	++*	++*
	GP	—	—	—	—	—
3	HM	—	—	—	+*	++*
	GP	—	—	—	—	—
4	HM	++	++*	++*	+*	—
	GP	++	—	—	—	—
5	HM	—	—	—	++*	++*
	GP	—	—	—	—	—
6	HM	—	—	+*	++*	+*
	GP	—	—	—	—	—
7	HM	—	—	—	—*	—*
	GP	—	—	—	—	—
8	HM	—	—	—	—	+*
	GP	—	—	—	—	—+
9	HM	—	—	—*	+*	+*
	GP	—	—	—	—	—
10	HM	—	—	—	++*	++*
	GP	—	—	—	+	+
11	HM	++	—	—	++	++
	GP	++	+*	+*	++*	++*
12	HM	++	+	—	++	++
	GP	++	+	++	+	—
13	HM	—	—	—	++	+
	GP	—	—	—	—	—
14	HM	—	—	—	—	—
	GP	—	—	—	—	—
15	HM	++	—*	+*	++*	++*
	GP	—	—	+*	++*	++*

— = no response, + = erythema, ++ = erythema with induration (20 min, 2 h and 6 h), ++ = erythema with induration or papules or both (24 and 48 h). * Skin biopsies were taken from these patch test reaction sites.

Patient 4 occurred at 2 h and in Patient 15 at 6 h. Patients without positive patch test reactions at 2–6 h showed an increase of eosinophils in the dermis at 24–48 h after testing. At this time, degranulated eosinophils were also seen. At 24–48 h after patch testing eosinophils were also present in the epidermis in eight patients (Fig. 1). These eosinophils were either scattered throughout the epidermis or preferentially located in the upper part of the epidermis. The total number of eosinophils in the epidermis at 24 h after patch testing varied from 5–22 per skin section. Some of the eosinophils in the epidermis were close to cells with a clear cytoplasm and a

TABLE 3. Numbers of eosinophils in the cellular infiltrate in the epidermis (E) and dermis (D) in skin biopsies taken at different times after patch testing of patients with atopic dermatitis

Patient no.	Allergen		Tests read at 20 min	2 h	6 h	24 h	48 h	Clinically involved skin	Control*
1	GP	E	—	. . .	. . .	+(—)	+(—)	—	—
		D	1(0)			11(6)	8(3)	2(1)	1(0)
2	HM	E	—	. . .	. . .	+(—)	+(—)	—	—
		D	0			10(4)	8(5)	3(1)	1(0)
3	HM	E	. . .	. . .	. . .	—	—	—	—
		D				6(4)	6(4)	1(0)	0
4	HM	E	—	—	—	—		—	—
		D	0	12(0)	7(5)	2(2)		3(0)	0
5	HM	E	. . .	. . .	. . .	+(nd)	—	+(nd)	—
		D				16(nd)	4(nd)	4(nd)	1(nd)
6	HM	E	. . .	. . .	—	—	. . .	—	—
		D			4(2)	4(2)		2(1)	0
7	HM	E	. . .	. . .	. . .	—	—	—	—
		D				0	0	0	0
8	HM	E	. . .	. . .	. . .	. . .	+	. . .	—
		D					4(nd)		1(nd)
9	HM	E	. . .	. . .	—	+	—	. . .	—
		D			0	4(nd)	2(nd)		0
10	HM	E	. . .	. . .	. . .	+(—)	—	—	—
		D				7(4)	5(4)	1(0)	0
11	GP	E	. . .	—	—	+(—)	+(—)	+(—)	—
		D		3(3)	12 12)	14(2)	10(5)	3(1)	1(0)
15	GP	E	. . .	. . .	—	—	+(—)	—	—
		D			14(10)	5(5)	12(9)	3(1)	0

In the dermis (D) the number of eosinophils and the number of EG2+ (activated) eosinophils (numbers in parentheses) are given as a percentage of all cells present. In the epidermis (E): + = eosinophils present, — = eosinophils absent, (—) = EG2 negative. nd = not done, * Patch test with control diluent at 24 h.

slightly lobed nucleus. This was observed 1–3 times in each skin section, especially in the upper part of the epidermis.

No infiltration of basophils or neutrophils was observed in the dermis at early reaction times or at 24–48 h after patch testing. At 2–6 h mast cell degranulation was present.

Immunostaining

Analysis of the dermal infiltrate revealed an increasing number of Leu 1 and OKT6 positive cells from 2–6 h to 48 h after patch testing. Leu 3a positive cells outnumbered Leu 2a positive cells. The T lymphocytes which infiltrated the epidermis at 24–48 h were mainly Leu 2a positive.

Staining of epidermal dendritic cells with anti-human IgE was positive in all skin sections investigated. The anti-human IgE staining of the dermal perivascular infiltrate increased from 2–6 h to 24–48 h after patch testing.

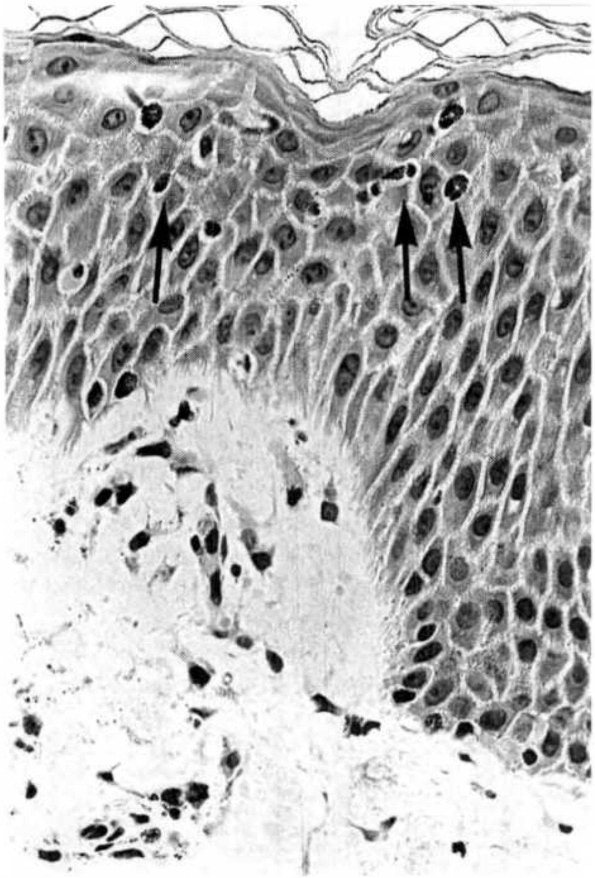

FIGURE 1. A skin biopsy taken 24 h after patch testing, showing an infiltrate of eosinophils (arrows) in the epidermis. (H&E; original × 400).

The results of immunostaining with EG2 antibody are given in Table 3. EG2 positive cells were present in the dermis at 2, 6, 24 and 48 h. The maximum number of EG2 positive cells was observed at 6 h after patch testing. Eosinophils present in the epidermis failed to stain with EG2 antibody, although staining with R-a-ECP antibody was positive. Double immuno-labelling studies with anti-human IgE and R-a-ECP antibodies and with OKT6 and R-a-ECP antibodies were negative. Double-labelled cells were not present. In the epidermis approximately 1–3 cells per skin section which stained with anti-human IgE were found adjacent to cells stained with R-a-ECP.

Electron microscopy

Electron microscopic studies demonstrated that some eosinophils present in the epidermis at 24 h, were near to or in close contact with Langerhans cells. These cells were not quantified. Extracellular granules derived from eosinophils were present, either scattered around these cells or between keratinocytes (Fig 2a and b).

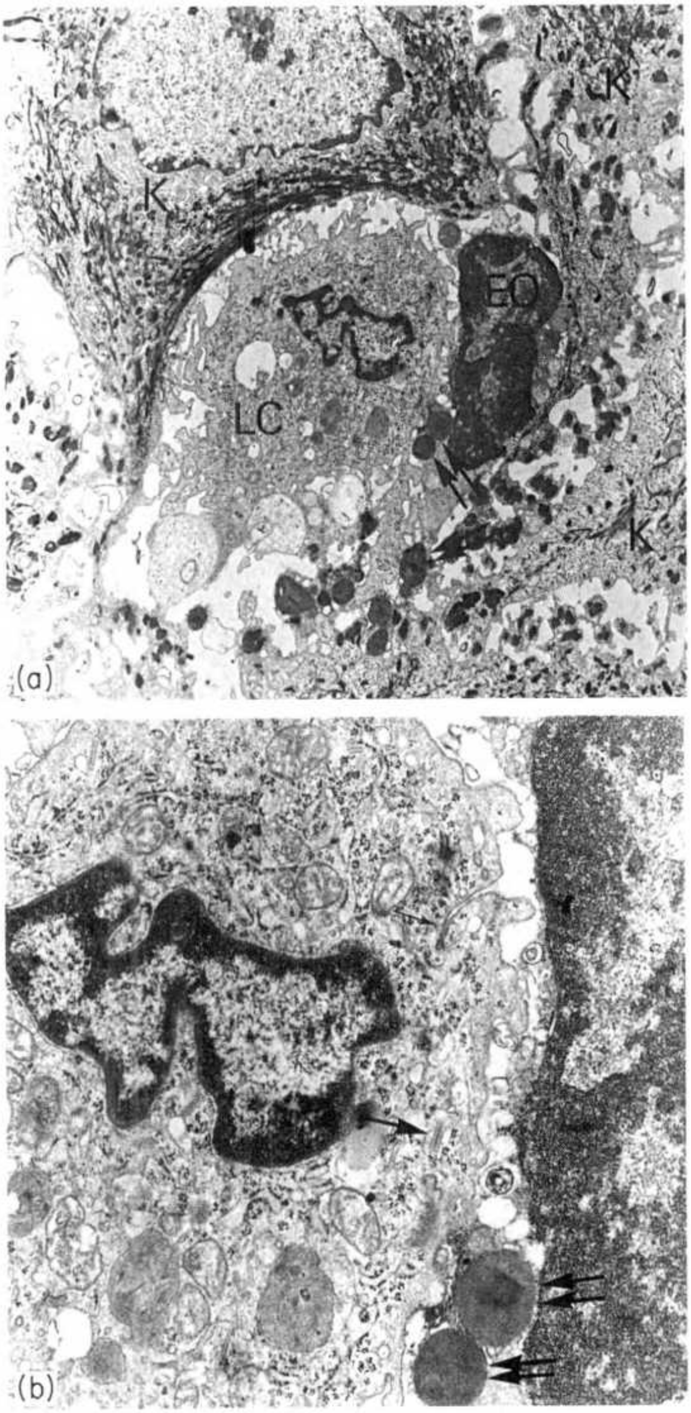

FIGURE 2. An epidermal Langerhans cell (LC) lying in close contact with an eosinophil (EO). K = keratinocytes, single arrows indicate Birbeck granules and double arrows indicate eosinophil derived granules. (a) (Original × 9,100) (b) (Original × 31,200).

DISCUSSION

Most patients with atopic dermatitis in the present study had positive reactions to HM allergen 24–48 h after patch testing. However, positive reactions at earlier times (20 min, and 2 h and 6 h) were often seen as well. Analysis of the dermal infiltrate revealed that eosinophils were present as early as 2–6 h after patch testing, and many were activated. Twenty four hours after patch testing, the number of activated eosinophils was less than at 6 h, whereas 48 h after patch testing the number was slightly greater. Furthermore, 24 h after patch testing, eosinophils penetrated into the epidermis where they were not activated. Interestingly, some of these eosinophils were in close contact with epidermal Langerhans cells (LC). These findings suggest that immediately after patch testing, some of the allergen penetrates the epidermis, binds to IgE molecules on mast cells in the dermis and induces an immediate type reaction; the mast cell releases an eosinophil chemotactic factor, and some of the infiltrating eosinophils become activated. This would explain the presence of EG2 positive eosinophils 2–6 h after patch testing. On the other hand, epidermal LC in patients with AD bear IgE molecules[8] and therefore, part of the epicutaneously applied allergens may bind to allergen specific IgE on LCs. After binding of allergens to epidermal LC two processes may be initiated. Firstly, since LC have a strong antigen presenting capacity,[9] a T lymphocyte proliferative response may be induced in the dermis. Secondly, an eosinophil chemotactic factor may be generated either directly or indirectly by epidermal LC.

At 6–24 h, the dermis was infiltrated by T lymphocytes. Activated T lymphocytes may attract eosinophils by the release of a lymphokine which may act as an eosinophil chemotactic factor.[12] Eosinophils infiltrating the dermis seem to be in an activated state and have lost part of their granular contents including intracellular ECP. Since ECP reduces lymphocyte proliferation *in vitro*,[19] eosinophils may, by the release of ECP, inhibit the allergen-induced lymphocyte response. On the other hand, at 24 h some of the eosinophils in the dermis migrate to the epidermis, where some of them may contact epidermal LC. This suggests that keratinocytes or epidermal LC or both from patients with AD release an eosinophil chemotactic factor upon challenge with an allergen. Keratinocytes may synthesize leukotriene B_4 which has chemotactic activity for neutrophils and eosinophils from normal individuals *in vitro*.[20] However, the absence of neutrophils in the epidermis and even in the dermis suggests the involvement of a factor with a selective chemotactic activity for eosinophils. Moreover, the cellular contact between eosinophils and LC suggests the release of an eosinophil chemotactic factor from LC. Since the significance of such contact is not yet clear, one can only speculate on the release of such a chemotactic factor after allergen binding to epidermal LC. Further investigations are needed to clarify this point. Taken together, our results suggest an active role for eosinophils in the mechanism involved in patch test reactions to inhalant allergens in patients with AD. The observation that these eosinophils even infiltrate the epidermis, where some of them may be in close physical proximity to IgE-bearing LC, suggests an interaction between eosinophils and LC. How eosinophils modulate the cellular response in these patch test reactions to allergens requires investigation.

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Malignant melanoma, benzoyl peroxide and acne: a pilot epidemiological case-control investigation

R.A.CARTWRIGHT, B.R.HUGHES* AND W.J.CUNLIFFE*

Leukaemia Research Fund, Centre for Clinical Epidemiology, University of Leeds, Leeds and * Dermatology Department, Leeds General Infirmary, Leeds, U.K.

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SUMMARY

A case-control study utilizing available medical records was carried out to investigate possible links between use of topical benzoyl peroxide for acne, and malignant melanoma. One hundred and fifty nine cases and 213 matched controls were used in the analysis which shows no significant association between the use of benzoyl peroxide and the occurrence of malignant melanoma, nor between the occurrence of acne and malignant melanoma.

Recent work has suggested that there may be a causal association between the past topical application of benzoyl peroxide (BP) and the subsequent development of skin tumours including malignant melanoma. This work was based on animal experimentation[1–3] and has been criticized.[4] Unfortunately, this inadequate animal data appeared in the media resulting in one of us (W.J.C.) receiving many concerned enquiries from both patients and dermatologists.

We report here an epidemiological case-control study carried out to investigate the possibility that human exposure to topical benzoyl peroxide might be hazardous.

METHODS

Case-control studies aim to contrast the past specific experience of cases with respect to features which might be hazardous (in this case benzoyl peroxide use and acne) with individuals of similar demographic identity (controls) who are chosen to represent the general population and who might have had the same past experience as the cases. In the present study controls were selected from the same general practitioner list as the cases to fulfil this criterion.

Correspondence: Dr R.A.Cartwright, Leukaemia Research Fund Centre for Clinical Epidemiology, 17 Springfield Mount, Leeds, LS2 9NG, U.K.

All cases of malignant melanoma of the skin, under the age of 45 years, seen between 1984 and 1986 inclusive were investigated. None of the patients or their relatives were approached and the study was exclusively based on hospital and general practitioner case records. In addition, general practitioners were asked to provide records for two control individuals of the same age and sex as each case, known not to have a malignant disease. The records of both groups were examined for details of acne, any prescribed skin medication and prolonged sunlight exposure. All information was recorded manually. If a case was deceased the general practitioners' records were obtained from the Family Practitioner Committee. Strict attention was paid to confidentiality, and anonymity of the records was maintained during analysis. The cases were obtained from the Yorkshire Regional Cancer Registry with the permission of the relevant consultants and were resident at an address in North Yorkshire, West Yorkshire or Humberside at the time of diagnosis.

Risk ratios and other statistics were calculated using the standard epidemiological programmes of Rothman and Boice.[5]

RESULTS

Two hundred and thirteen cases of melanoma were identified in the relevant age group and time period. Of these, 159 cases (75%) were investigated further. Of those not investigated, 32 (15%) were not traced within the Yorkshire Health Region; the study was not extended beyond the boundaries of the region. The remaining 22 (10%) were not included either because of the late arrival of notes after the end of the study period, because the patient had joined the armed forces or because the general practitioner has refused assistance (eight cases).

The cases analysed were evenly distributed throughout the health districts of the Yorkshire Region with the exception of South Humberside where a disproportionate number of cases were not traced.

TABLE 1. Numbers of malignant melanoma cases and controls with a recorded history of acne

	No. of individuals with acne			
	Cases		Controls	
Age range (years)	Male	Female	Male	Female
15–24	5	18	9	23
25–34	1	4	3	6
35–44	0	1	0	5
Total	6	23	12	34

Risk ratio between past acne and malignant melanoma = 1·1; 95% confidence limits = 0·7–1·9, $P = 0{\cdot}39$.

TABLE 2. Numbers of malignant melanoma patients and controls who had used benzoyl peroxide

	No. of individuals using benzoyl peroxide			
	Cases		Controls	
Age range (years)	Male	Female	Male	Female
15–24	0	2	4	8
25–34	1	2	0	2
35–44	0	0	0	1
Total	1	4	4	11

Risk ratio between use of benzoyl peroxide and malignant melanoma = 0·5; 95% confidence limits = 0·2–1·5, $P = 0{\cdot}17$.

Controls were provided by the general practitioners using a random matching procedure. Two controls were sought for each case; these were of the same sex as the case, with dates of birth within 3 years of that of the case and from the list of the same general practitioner. Occasionally two suitable controls could not be found or the general practitioner was unwilling to provide two controls. Thus, notes from 264 matched controls were studied, giving a case:control ratio of 1:1·7.

There were 51 males and 108 females in the case group and 81 cases (51%) were in the age range 35–44 years. The site of the melanoma was on the head or neck in seven males and in five females, none of whom had used benzoyl peroxide. A further seven cases had malignant melanoma on the back or chest and also had had recorded acne.

Table 1 contains details of numbers of cases and controls with recorded acne and Table 2 recorded benzoyl peroxide use, with risk ratios and 95% confidence limits of the estimates. The independent analyses of each factor indicate no significant association of either factor with melanoma. None of the risk estimates was statistically significant; neither the slight excess risk with respect to past acne nor the lower risk with respect to past benzoyl peroxide use.

No significant excess of benzoyl peroxide use was found amongst cases with melanoma on the head, neck or back.

The nature of the study was such that no data were available with regard to sun exposure.

DISCUSSION

This study has some limitations. It was relatively small and a major flaw is that there was no direct approach of the patients to ask whether or not they had had acne which was treated by their general practitioner, or whether they had purchased benzoyl peroxide without prescription. Fifteen percent of the general population have clinical acne, but 85% have 'physiological' acne[6] and it is this latter group who may buy benzoyl peroxide without prescription. Because much benzoyl peroxide is purchased without prescripton and much acne is unreported, the results do not necessarily reflect the true prevalence of these events in the case and control samples. Benzoyl peroxide was prescribed exclusively for the acne sufferers, in this study.

It is very possible, however, that the more severe acne is represented in this study because that would be most likely to be brought to the attention of the medical practitioner, and such subjects use much more topical therapy than subjects with no clinical acne. What is more important, however, is that for each general practitioner, the controls are likely to be similar to the cases in this respect. It seems unlikely that individuals in the case group would have presented more or less frequently with severe acne than the controls and so the essential element of a case-control approach, like record being compared with like record, is adhered to.

Our results are in agreement with those of Beral *et al.*[7] and Elwood *et al.*[8] whose studies of melanoma and acne demonstrated no statistically significant association. In this latter study newly diagnosed cases of malignant melanoma were interviewed and compared with age and sex matched controls. These authors also obtained information about topical treatment. Only 24% of subjects reported having had acne and very few mentioned the use of benzoyl peroxide containing compounds.

The present small study indicates that there is no evidence that acne sufferers (who may have used benzoyl peroxide without prescription) or individuals prescribed benzoyl peroxide, have an increased risk of malignant melanoma.

ACKNOWLEDGMENTS

We would like to thank the consultants and general practitioners in the Yorkshire Health Region for their assistance in this confidential enquiry, and also the Yorkshire Regional Cancer Registry. Grateful thanks also to Cherry Barlett, June Greatrex, Jackie Adams and Jill Collins who were involved with the work and its preparation for publication.

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Manifestation of familial porphyria cutanea tarda after childbirth

L.MALINA AND C.K.LIM*

Department of Dermatology, Faculty of Medical Hygiene, Charles University, Prague 10, Czechoslovakia and
*Division of Clinical Cell Biology, Clinical Research Centre, Watford Road, Harrow, Middlesex, U.K.

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SUMMARY

We report the case of a woman with hereditary porphyria cutanea tarda which manifested 3 weeks after she gave birth to her second child. The mother of the patient had also been diagnosed and treated for porphyria cutanea tarda. Reduced red cell uroporphyrinogen decarboxylase activity was found in the patient, the new-born child and the patient's mother. Normal enzyme activity was found in the patient's first child.

Porphyria cutanea tarda (PCT) is an aetiologically diverse group of disorders with reduced uroporphyrinogen decarboxylase activity. Sporadic and familial forms occur, differing according to whether the enzyme defect is in the liver only or in all tissues.[1,2] Both sporadic and familial PCT are often precipitated by factors such as chronic alcoholism, iron overload and oestrogen therapy.[3]

The porphyrinogenic effect of oestrogens can occur in men being treated for prostatic carcinoma and in women given oestrogens for gynaecological therapy or oral contraception. Evidence for the influence of hormone levels on the precipitation or exacerbation of PCT during pregnancy, however, is inconclusive.[4–7] Furthermore, none of the patients investigated in these studies had familial PCT.

We report here a case of familial PCT which manifested in the puerperal period. The patient's mother, who had three children and no abortions or miscarriages, has also been diagnosed and treated for PCT. Her father and brothers were not affected.

CASE REPORT

A 29-year-old woman presented with bilateral blisters and erosions on the dorsum of the fingers and hands. These lesions had first appeared during the third week after she gave birth to her second child. The patient's history included coeliac disease in early childhood and splenectomy

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Correspondence: Dr. L.Malina, Faculty Hospital, Dept. Dermatology, Šrobárova 50, 100 34 Prague 10, Czechoslovakia.

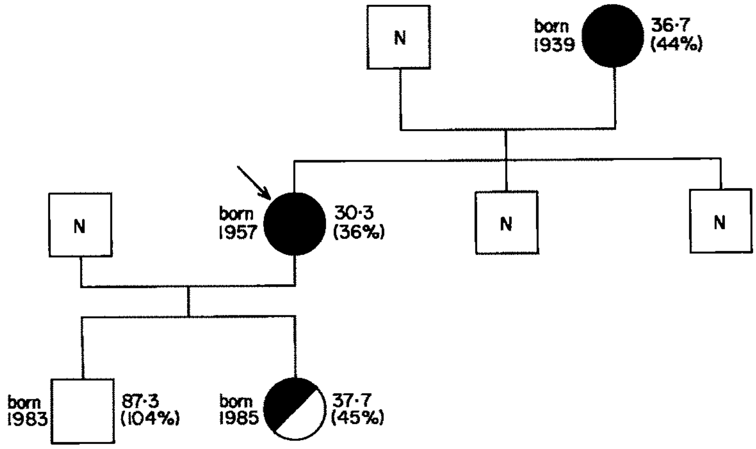

FIGURE 1. Family pedigree, and results of erythrocyte uroporphyrinogen decarboxylase (URO-D) assay. Values are URO-D activity expressed as nmol coproporphyrinogen III formed/ml RBC/h. Values in parenthesis are URO-D activities expressed as a percentage of the mean for normal controls. ●: manifest PCT; ◐: latent PCT; ▣: URO-D not measured. Arrow indicates propositus.

when she was 9 years old, following an injury. She had had 2 pregnancies (the first in 1983), both delivered by caesarian section. She had never taken oral contraceptives. On admission the urinary porphyrin level was markedly raised (coproporphyrin 371·0 μg/day; uroporphyrin 2346·0 μg/day). The urinary porphyrin excretion pattern, analysed by high-performance liquid chromatography, was typical of PCT with elevated levels of uroporphyrin and heptacarboxylic porphyrin.[8] The faecal porphyrin profile was also consistent with PCT, with increased excretion of heptacarboxylic porphyrin and isocoproporphyrin. Haematology and urine analysis were normal. Serum bilirubin was 31·0 μmol/l; alanine aminotransferase 1·26 μmol/s and aspartate aminotransferase 0·76 μmol/s. Plasma gamma-globulin was 0·28 g/l. Liver biopsy revealed only moderate siderosis and focal large-droplet steatosis.

Familial PCT was confirmed by measuring red cell uroporphyrinogen decarboxylase activity using purified pentacarboxylic porphyrinogen III as the substrate.[9] Enzyme activity was 30·3 nmol coproporphyrinogen III formed/ml RBC/h. This represents a decrease to less than 40% of the value in normal controls (Fig. 1). A similar enzyme defect was detected in the patient's mother and in her newborn child (Fig. 1). Activity of the enzyme was normal in the patient's first child. Enzyme activities in the patient's brothers and father were not measured as they were found by porphyrin analysis not to have PCT.

The patient was treated with low-dose chloroquine, with significant subjective and objective improvement.

DISCUSSION

Urine and faecal porphyrin excretion patterns together with inhibition of red cell uroporphyrinogen decarboxylase activity provide definitive evidence of familial PCT. Since the patient had not been treated with drugs known to precipitate PCT we conclude that the manifestation of familial PCT was induced by endogenous hormonal factors. To the best of our knowledge, this is the first report of the manifestation of familial PCT due to this condition. We have seen a similar case, but of exogenous origin, in a male patient treated with diethylstilbestrol for prostatic cancer.[10] The question arises as to why our patient's first pregnancy did not result in her PCT becoming manifest. The pattern of genetic transmission may possibly have played a role here.

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The suitability of sunglasses used by patients following ingestion of psoralen

H.MOSELEY, N.H.COX* AND R.M.MACKIE*

Department of Clinical Physics and Bio-Engineering, West of Scotland Health Boards, Glasgow and *University Department of Dermatology, Western Infirmary, Glasgow, U.K.

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SUMMARY

We have investigated the types of eyewear which patients on photochemotherapy have obtained, and determined their suitability for providing adequate protection. Fifty-eight pairs of sunglasses from 53 patients were assessed. Spectral transmission was measured between 200 nm and 800 nm at 10 nm intervals. Thirty-eight pairs of glasses (66%) were found to be satisfactory. Particularly high UV transmission was recorded in 11 cases (19%), highlighting the need for increased awareness of the problem of ensuring that suitable protective eyewear is used.

The possibility of inducing cataract in patients on long-term photochemotherapy (PUVA) has been widely recognized from work on experimental animals.[1] During treatment, patients are provided with goggles which absorb the incident ultraviolet radiation. From the time of ingestion of psoralen and throughout the remainder of the day, patients are instructed to avoid unnecessary exposure to sunlight and to wear UV-blocking sunglasses or eye shields when outdoors. It has been shown that there is a wide variation in the UV absorbing power of sunglasses and that most, but not all types of polarizing material provide substantial protection against solar UVA.[2]

The purpose of the present study was to examine the protective properties of sunglasses in regular use by our PUVA patients in order to establish the level of protection afforded to the patients in practice.

METHODS

A total of 58 pairs of sunglasses from 53 patients were investigated. The name of the manufacturer or supplier was recorded when known and it was noted whether or not the lens was composed of a polarizing material. The ultraviolet and visible radiation transmission properties of the eyewear were measured as described elsewhere.[3,4] In the waveband 200 nm to

Correspondence: Dr H. Moseley, Department of Clinical Physics and Bio-Engineering, West of Scotland Health Boards, 11 West Graham Street, Glasgow G4 9LF, U.K.

TABLE 1. Transmission limits for 'satisfactory' sunglasses

Wavelength (nm)	Transmission limit (%)
390	10
380	5
370	2
360 and below	1

520 nm, a deuterium discharge lamp and a UV double grating spectroradiometer were used and, between 500 nm and 800 nm, a tungsten halogen lamp and an International Light IL 700A spectroradiometer were used. Readings were taken at 10 nm intervals and a computer program enabled transmission to be calculated at each wavelength and a plot of transmission against wavelength obtained for each pair of sunglasses. In the case of eyewear containing light-sensitive filters, UV readings were taken with simultaneous exposure from the deuterium lamp and the tungsten lamp.

TABLE 2. Results of transmission measurements carried out on 58 pairs of sunglasses used by patients undergoing PUVA therapy

	Type of sunglasses	Polarizing[1]	Number
Satisfactory	Polaroid	P	18
	Unknown	P	9
	Boots	P	2
	Unknown	N	7
	Aoline Polaris	N	1
	Boots	N	1
Unsatisfactory	Unknown	N	3
	Unknown coated spectacle	N	2
	Reactolite coated spectacle	N	1
	Boots graduated shading[2]	N	1
	Unknown graduated shading[2]	N	1
	Unknown goggle	N	1
	Reactolite[3,4]	N	3
	Unknown[4]	N	3
	Polaroid, graduated shading[2,4]	N	2
	Polaroid[4]	N	1
	Zeiss Umbral[4]	N	1
	Unknown coated spectacle[4]	N	1

Notes:
(1) P—polarizing, N—non-polarizing.
(2) Transmission through middle of filter.
(3) Close proximity between lamp and glasses may have caused temperature rise and produced increased transmission.
(4) Unusually high UV transmission.

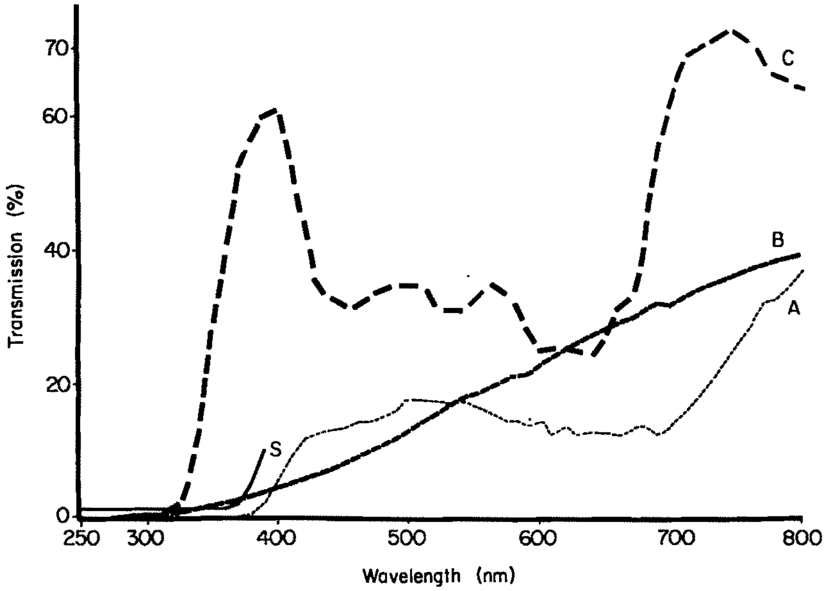

FIGURE 1. Spectral transmission curves of three pairs of sunglasses used by PUVA patients. A: 'satisfactory'. B: 'unsatisfactory'. C: 'unsatisfactory' with unusually high UV transmission. S is the transmission limit for 'satisfactory' eyewear.

Sunglasses were arbitrarily defined as 'satisfactory' if they were within the transmission limits listed in Table 1. Eyewear which fell outside these limits was recorded as 'unsatifactory' and, within this group, that with particularly high UV transmission was identified.

The limits specified in Table 1 were based on the following criteria: (1) Glasses should provide adequate protection. It would seem reasonable to seek protection at wavelengths shorter than 380 nm, but, in the absence of direct experimental data, a precise definition of 'adequate protection' cannot be given. (2) Suitable glasses should be readily available. The fact that 66% of glasses tested fulfilled the specified conditions proves that such eyewear is readily available. (3) Glasses should be cosmetically acceptable. This increases the likelihood of compliance. The transmission limits were formulated in such a way as to allow the filters to be uncoloured. This helps with cosmetic acceptability and is important in cases when the patients require correct colour perception to carry out their normal occupation.

RESULTS

The results are presented in Table 2. Thirty-eight pairs of glasses (66%) were found to be 'satisfactory', including 13 clip-ons. Twenty-nine of the 38 'satisfactory' glasses had polarizing filters. All of the 20 'unsatisfactory' glasses had non-polarizing filters and included all four pairs tested which had graduated shading (dark at top, light at bottom). Results for these refer to transmission through the middle of the filter. All four coated spectacles examined were 'unsatisfactory' as were the four Reactolite light-sensitive sunglasses. In the latter, the glasses did not darken significantly although they were exposed to high levels of visible radiation. It may

TABLE 3. Transmission at wavelengths below 400 nm for the three pairs of sunglasses shown in Figure 1. Transmission limit for satisfactory glasses included for comparison.

Wavelength (nm)	Transmission (%). Sunglasses A	B	C	Transmission limit for 'satisfactory' glasses (%)
390	2·3	3·9	61·0	10·0
380	0·4	3·2	57·1	5·0
370	0·0	2·6	52·8	2·0
360	0·0	2·1	42·7	1·0
350	0·0	1·8	28·3	1·0
340	0·0	1·3	13·2	1·0
330	0·0	1·0	3·8	1·0
320	0·0	0·7	0·5	1·0
310	0·0	0·4	0·0	1·0
300	0·0	0·3	0·0	1·0
290	0·0	0·0	0·0	1·0

be that close proximity between lamp and glasses caused a temperature rise which produced increased transmission.

Examples of spectral transmission curves are shown in Figure 1 and values for transmission at wavelengths below 400 nm are given in Table 3. This illustrates the range of spectral transmission encountered. Figure 1 includes an example of a transmission curve from a 'satisfactory' pair of glasses; these were made by Polaroid and contain polarizing filters. The figure also illustrates the spectral transmission of two pairs of glasses of unknown make classified as 'unsatisfactory'. One of these satisfied our criteria at 380 nm and 390 nm but exceeded the transmission limits at 370 nm and 360 nm down to 340 nm; the other pair are notable in having unusually high UV transmission.

DISCUSSION

The importance of the present study is that we examined protective eyewear which patients were in fact using. It thus provides the information which is often lacking in assessments of possible ocular complications resulting from long-term PUVA therapy. Patients were all instructed to buy 'polaroid-type' sunglasses. Yet only 29 out of the 58 glasses tested contained polarizing filters.

In the United States, a sub-committee of the Task Forces of Psoriasis and Photobiology have issued revisions of their original advice on eye protection.[5] Their new recommendations are that patients wear UVA-blocking wrap-around glasses during the course of the day, after ingestion of the psoralen. They also recommend the wearing of UVA-blocking glasses during the following day. In the standard which we have promulgated, we have not specified wrap-around glasses. This was done with a view to improving compliance. In addition to the direct transmission of ultraviolet radiation through the filter, there will be a certain amount of leakage around the sides, top and bottom of the glasses, some of which will reflect off the back surface of

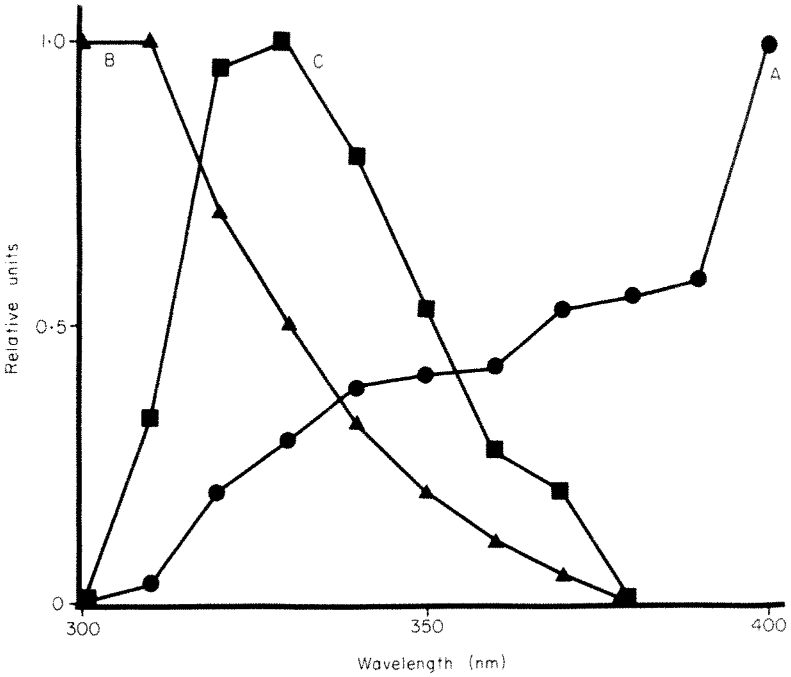

FIGURE 2. An estimate of the relative effectiveness of different wavelengths for possible PUVA-induced cataract (Curve C). This curve is the product of the spectral irradiance incident on the lens (Curve A) and the absorption spectrum of 8-MOP (Curve B). Each of the curves has been normalized to unity at its maximum value between 300 nm and 400nm.

the filter. Further investigation is required to determine the importance of this leakage radiation.

To our knowledge, no action spectrum has been published for the induction of lenticular opacities by PUVA in any species. It is possible, however, to provide an estimate of the relative effectiveness of different wavelengths based on existing published data. The irradiance incident on the lens can be calculated from the spectral irradiance of daylight from sun and sky[6] and the transmission characteristics of cornea and aqueous humour.[7] The absorption spectrum of 8-MOP has been published.[8] Although the daylight irradiance and ocular transmission combine to produce an incident irradiance on the lens which increases with wavelength in the UVA range (Fig. 2, curve A), the absorption of 8-MOP decreases as the wavelength increases (Fig 2, curve B). The product of the values for incident irradiance and 8-MOP absorption at each wavelength can be used to give an estimate of the relative spectral effectiveness for a possible PUVA-induced cataract (Fig. 2, curve C). Although the tentative nature of this combined spectrum must be stressed, it suggests that the most important wavelengths for cataract formation in patients having PUVA treatment may be centred around 330 nm. Unfortunately, without direct experimental data, an action spectrum in absolute units cannot be determined.

Lerman *et al.*[9] demonstrated that free 8-MOP can be detected in a human lens for at least 12 h following oral ingestion of a single therapeutic dose (0·75 mg/kg). They also showed, in rats, that free 8-MOP tends to diffuse out of the lens within 12–24 h provided the animals are kept in the dark. If the rats are exposed to light, photo-addition products are generated which may be retained permanently within the lens. Thus, if patients protect their eyes from exposure to light for 12–24 h following ingestion of 8-MOP ocular complications of PUVA should be prevented.

Cases of cataract formation have been reported in patients undergoing PUVA therapy[10,11]. Hammershoy and Jessen[12] studied cataract formation in 96 patients treated with PUVA. Patients were advised to wear protective glasses for 24 h after treatment. The occurrence of cataract in the latter study[12] was found to correspond with that expected in the general population. In another study of 78 patients[13] small lens opacities appeared in three patients, two of whom had not worn their protective glasses for 24 h after each treatment, as recommended.

One extensive follow-up study showed that there was no increase in the risk of symptomatic cataract in patients who had been treated with PUVA and who wore UVA protective eyewear.[14] In another recent follow-up study,[15] no patients were found to have any significant changes in retinal function tests designed to detect sub-clinical deterioration in visual function.

Although few ocular side-effects have been reported, transient visual field defects in patients receiving PUVA therapy have been observed[16] and these defects appear to be related to the dosage of 8-MOP. In their review of long-term risks of PUVA, Farber *et al.*[17] observe that, although late ocular complications may be prevented by suitable eye protection, there is concern over possible lack of patient compliance.

The results of the present investigation supplement the studies of Wennersten[18] and Davey *et al.*[2] who examined types of sunglasses available on the open market. As a practical point, it is worth noting that not all Polaroid brand name glasses contain polarizing lenses. This is important since we are currently recommending the use of polarizing glasses to our patients. A simple test of the polarizing nature of a lens is to hold the unknown filter against a known polarizing filter and rotate the unknown filter. The unknown filter is polarizing if, on a complete 360° rotation, transmitted light undergoes two intensity modulations from dark to light. To this end, it would be useful for dermatology departments to obtain a pair of polarizing glasses and use one of the lenses to perform the above test on patients' eyewear. Moreover, since reflected light is polarized to a certain extent, viewing light reflected from a shiny surface through a polarized filter also undergoes intensity modulation on rotating the filter; another quick check is to view the liquid crystal display of a digital watch through the rotating filter.

A brief comment should be made concerning the apparently poor performance of the Reactolite light-sensitive glasses. These contain material which darkens on exposure to visible radiation and, according to the manufacturer, attenuation of light should extend into the ultraviolet region. This did not happen on exposure to the deuterium arc lamp alone. Since this might have been due to inadequate stimulation by visible light, glasses with light-sensitive lenses were tested with a supplementary high brightness tungsten halogen lamp. However, transmission was not appreciably altered and so we are unable, at this stage, to endorse their use. A possible explanation for our results could be the heating effect of the tungsten lamp since the materials used are known to be temperature-sensitive as well as light-sensitive.

It is the practice of some to check sunglasses by using radiation from the opened door of the PUVA box and a UVA-meter. Dermatologists should be cautioned against placing undue reliance on such a technique. What is being measured in this case is the total transmission of the emitted radiation of the UVA lamps over the wavelength interval defined by the spectral sensitivity of the UVA meter. This will give rise to considerable variation depending on the lamp–detector combination and will certainly not correspond with the derived spectral effectiveness for possible daylight-induced lenticular opacities shown in Figure 2. The use of the PUVA box and UVA meter does not have the sensitivity of the spectral analysis reported here and, at best, would be capable of picking out only the poorest sunglasses.

The present study provides factual information on the types of eye protection worn by patients. It is clear from our results that continued guidance must be given to patients on the wearing of suitable sunglasses. Also, it raises the question of whether criteria of acceptability should be drawn up and eyewear examined objectively against such a standard.

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Pharmacological modulation of cold-induced pain in cutaneous leiomyomata

C.B.ARCHER, S.WHITTAKER AND M.W.GREAVES

St John's Hospital for Diseases of the Skin, Leicester Square, London U.K.

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SUMMARY

In two patients with painful cutaneous leiomyomata, induction of pain by the application of an ice cube allowed assessment of a number of topical and systemic treatments aimed at reducing or preventing the pain. In one patient the alpha-adrenoceptor blocker, phenoxybenzamine alleviated cold-induced pain. In the second patient, topical 9% hyoscine hydrobromide (an anticholinergic agent) decreased pain induced by the ice cube, but was not helpful in reducing lesional pain due to cold weather.

Cutaneous leiomyomata are benign smooth muscle tumours which are derived from the arrectores pilorum muscles of the skin. They may occur as solitary or multiple lesions and are frequently painful. Pain can occur spontaneously or in response to pressure or changes in temperature.[1] We report here the pharmacological treatment of two cases of cutaneous leiomyomata in which the predominant symptom was pain, occurring particularly in cold weather.

CASE REPORTS

Case 1

A 27-year-old man, from Bangladesh, was seen in 1984, having developed skin-coloured nodules on the left upper arm 9 years previously. The lesions gradually became widespread, involving the arms and trunk, and some were pedunculated, resembling neurofibromata. The tumours were associated with pain, occurring either spontaneously or induced by low environmental temperatures, exercise (particularly in cold weather) and direct pressure.

One nodule on the abdomen was exquisitely tender, having increased in size during the previous 3 months (Fig. 1). The possibility of malignant change was considered and the nodule was surgically removed. The histology of this lesion was that of a well-defined dermal tumour,

Correspondence: Dr Clive B. Archer, St John's Hospital for Diseases of the Skin, 5 Lisle Street, Leicester Square, London WC2H 7BJ, U.K.

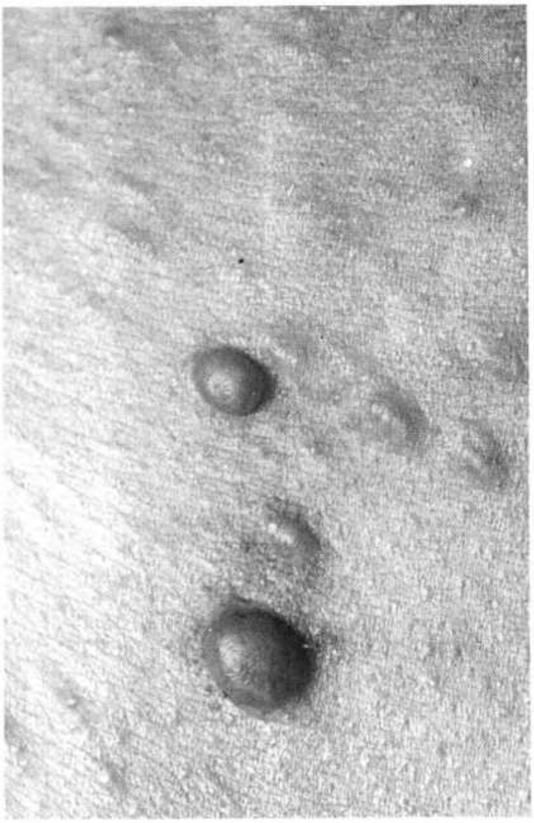

FIGURE 1. Case 1. Leiomyomata on the abdomen. The lower lesion had recently enlarged and was very painful.

comprising spindle cells with characteristic long, thin 'cigar-shaped' nuclei (Fig. 2). There was no evidence of cellular atypia to suggest malignant change. Excision biopsies of a number of other lesions were also diagnostic of leiomyoma. The nodules were too numerous to consider surgical removal of all of them, and previous treatment with oral analgesics had been unsuccessful. Some of the lesions had been treated previously with topical capsaicin, with the aim of depleting the nerve terminals of substance P, so rendering them unresponsive to painful stimuli. However, the application of capsaicin, in itself, was painful and did not relieve the subsequent development of pain in the leiomyomata. In addition, a diagnosis of maturity-onset diabetes mellitus had been made in 1983 and the patient had the clinical features of acanthosis nigricans in the flexures, particularly the axillae.

Case 2.

A 46-year-old woman, seen in 1985, had developed an erythematous nodule on the dorsum of the left wrist some 20 years previously. The tumour had slowly increased in size to form a group of nodular lesions extending around the wrist (Fig. 3), but had been no more than a cosmetic nuisance until 4 years previously, when, following a diagnostic biopsy, she had been troubled by recurrent pain. The pain sometimes occurred spontaneously but was particularly noticeable in

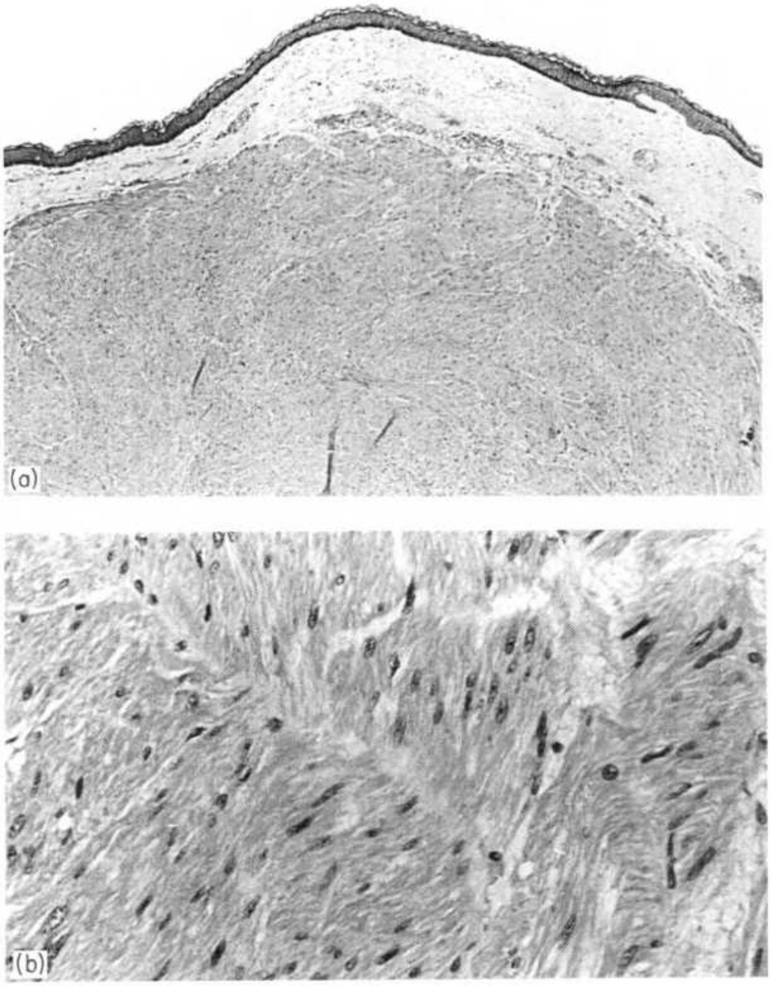

FIGURE 2. Sections of a leiomyoma from Case 1, showing (a) a well-circumscribed dermal tumour (H&E; original × 50) and (b) proliferation of spindle cells containing characteristic 'cigar-shaped' nuclei (H&E; original × 250).

cold weather. Previous treatment with simple analgesics had been unhelpful. Histopathological examination of the diagnostic biopsy revealed a dermal spindle cell tumour, consistent with leiomyoma. A hysterectomy had been performed in 1976 for menorrhagia related to fibroids.

ASSESSMENT OF POTENTIAL PHARMACOLOGICAL TREATMENTS

In both patients, we found that pain could be induced in the leiomyomata by the application of an ice cube, wrapped in gauze, to the overlying skin. The patients were stabilised on oral therapy for 24 h or on topical therapy for 4 h prior to assessment. In a standardized environment, we measured the time taken from the application of the ice cube to the onset of pain. At this point, the ice cube was removed and the time taken for the pain to resolve was recorded. On the

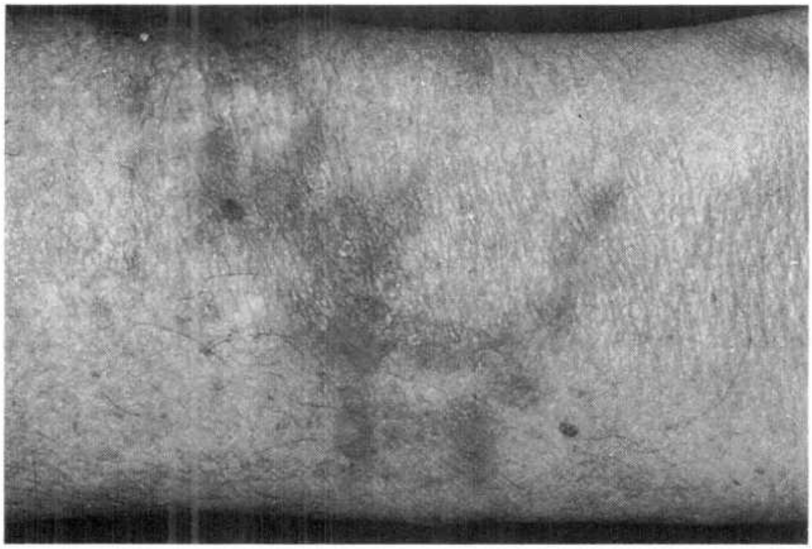

FIGURE 3. Case 2. Extensive leiomyoma comprising a group of erythematous nodules on the dorsum of the wrist.

TABLE 1. Drugs assessed using the ice cube test

Drug	Mode of administration	Mode of action
Local:		
1% phentolamine solution	topical	alpha-adrenoceptor blockade
9% hyoscine hydrobromide solution	topical	anticholinergic
Nitroglycerine paste and Transiderm Nitro plaster®	topical	smooth muscle relaxation
2% lignocaine gel	topical	local anaesthetic
Systemic:		
glycerol trinitrate	sublingual	smooth muscle relaxation
phenoxybenzamine	oral	alpha-adrenoceptor blockade
nifedipine	oral	calcium channel blockade
paracetamol	oral	analgesic

assumption that cold-induced pain in cutaneous leiomyomata is related to smooth muscle contraction, the 'ice cube test' was used to assess the effects of a number of topical and systemic treatments (Table 1) in comparison with placebo treatments.

In Case 1, phenoxybenzamine, 10 mg twice daily, increased the time lag for the development of pain from 30 s to 50 s and reduced the time taken for the pain to resolve from 180 s to 105 s. Placebo treatment did not alter these times. Phenoxybenzamine was subsequently found to be clinically useful in this patient, despite the well recognized side effect of retrograde ejaculation. With the onset of winter, however, cold-induced pain again became a problem. Symptomatic relief was achieved at the higher dose of 10 mg t.d.s. but this was accompanied by dizziness and nausea, necessitating a reduction in the dosage. The other treatments listed in Table 1, including nifedipine (10 mg t.d.s.), were ineffective in reducing ice cube-induced pain.

To assess the reproducibility of the ice cube test the time between application of the ice and the development of pain, and the time taken for the pain to resolve, without treatment, was measured in Case 1 on three different occasions. The mean times ± SD were 31·7 ± 2·9 s and 160·0 ± 22·9 s, respectively.

In Case 2, topical 9% hyoscine hydrobromide was of some benefit in relieving ice cube-induced pain and its effect lasted for approximately 6 h. Paracetamol was not helpful alone but, assessed by the ice cube test, did appear to augment the effects of topical hyoscine. The patient was advised to apply the hyoscine solution three times a day, but there was a problem of compliance and she used the solution once a day only. With this regimen, the cold weather again induced an uncomfortable, prolonged, aching pain. The other pharmacological treatments, including phenoxybenzamine and nifedipine, were ineffective. However, the lesional pain has subsequently been improved by liquid nitrogen cryotherapy.

DISCUSSION

The use of an ice cube proved to be a reliable and consistent method of inducing pain in the cutaneous leiomyomata in these patients, and allowed the objective assessment of various pharmacological agents. Oral phenoxybenzamine (Case 1) and topical hyoscine (Case 2) increased the time lag to the onset of pain and reduced the duration of pain, when compared with placebo treatments.

Solitary painful cutaneous leiomyomata can be excised surgically, but the rate of recurrence or development of adjacent satelite tumours can be as high as 50%,[2] and surgical removal of each tumour is not feasible in patients with multiple leiomyomata. Furthermore, an initial diagnostic biopsy in Case 2 appeared to precipitate the development of pain in the tumour. Regarding the question of potential malignant change, present evidence suggests that the rare superficial leiomyosarcomata do not arise in pre-existing leiomyomata.[2]

A number of systemic agents have been used to control or suppress pain in cutaneous leiomyomata. Phenoxybenzamine has been known to be beneficial for some time,[3] and, more recently, subjective improvement was reported with oral nifedipine.[4] This drug inhibits the trans-membrane influx of calcium ions, essential for contraction in cardiac and smooth muscle. However, we did not observe a response to nifedipine in our two cases.

Whether the pain in cutaneous leiomyomata is due to smooth muscle contraction[2] or to compression of nerve bundles within large tumours, is unclear.[1,5] Arrectores pilorum muscle contraction is mediated by the sympathetic nervous system. Noradrenalin secreted by post-ganglionic fibres activates alpha-adrenoceptors and smooth muscle contraction ensues. The parasympathetic nervous system has no effect on these smooth muscles, although all preganglionic fibres of the autonomic nervous system are cholinergic. Smooth muscles also contain afferent nerve endings which are stimulated by pressure or tension, giving rise to pain which is mediated via cholinergic fibres.[6] The existence of this dual innervation of the arrectores pilorum muscles is supported by histochemical studies of arrectores pilorum muscles from normal skin and from cutaneous leiomyomata.[7] The response of one of our patients to an alpha-adrenoceptor blocker and of the other to a topical anticholinergic agent might be explained by this dual innervation of the arrectores pilorum muscles. The subsequent improvement following local cryotherapy in Case 2 was presumably due to destruction of these nerve fibres.

We believe that the ice cube test offers a convenient, reliable and objective method for assessing potential treatment of painful cutaneous leiomyomata.

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A case of a lymphoproliferative disorder: Usefulness of DNA analysis in diagnosis

S.IDEYAMA, T.TANAKA, M.AKAGI, Y.HORIGUCHI, Y.MIYACHI AND S.IMAMURA

Department of Dermatology, Faculty of Medicine, Kyoto University, Japan

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SUMMARY

We describe a patient in whom a suppressor/cytotoxic T cell lymphoma was suspected on the basis of histology and immunohistochemistry. DNA analysis of specimens from three different lesions, using restriction enzymes *Eco* RI and *Bam* HI, showed the same rearrangement of the T cell receptor β-chain genes. We consider this technique to be of value in the early diagnosis of malignant lymphoma when differential diagnosis from other lymphoproliferative disorders is difficult with conventional methods.

The differential diagnosis of malignant lymphomas from benign lymphoproliferative disorders is made mainly by histology. However, it is sometimes difficult even for expert histopathologists.[1] Additional methods using monoclonal antibodies, still leave some problems. For example, in cases when malignant lymphoid cells are seen intermixed with inflammatory lymphocytes, there can be uncertainty as to which cells are the major component of the tumour. In the present study, we applied DNA analysis to make a diagnosis in a lymphoproliferative disorder which appeared to be a suppressor/cytotoxic T cell lymphoma on histology and immunohistology.

CASE REPORT

An 81-year-old man was seen at Kyoto university hospital in January 1987 complaining of several nodules on his trunk. Six months earlier, he had noticed a small reddish nodule on the left side of his abdomen which slowly enlarged up to 3 cm in diameter and disappeared spontaneously within the following 4 weeks. In December 1986, he found several similar nodules. On examination, he had four reddish asymptomatic nodules (1–2 cm in diameter) on the left side of his abdomen (Fig. 1). Each nodule had a well-circumscribed edge and was surrounded by slight erythema. His general health was good and no lymphadenopathy was

Correspondence: Dr Shin Ideyama, Department of Dermatology, Faculty of Medicine, Kyoto University Shogoin, Sakyo-ku, Kyoto, 606 Japan

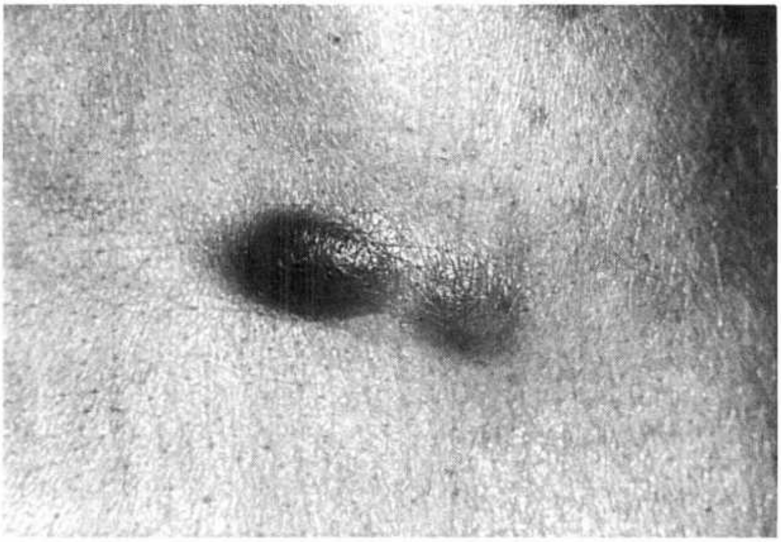

FIGURE 1. Two nodules on the left side of the abdomen, 1 cm and 2 cm in diameter respectively.

detectable. His past and family histories were unremarkable. The following tests were normal or negative: full blood count, differential leukocyte count, HTLV-I antibody titre, erythrocyte sedimentation rate, hepatic function, urinalysis, chest X-rays, ^{67}Ga and ^{99m}Tc-scintigraphy, lymphangiography and CT scans. A peripheral blood smear revealed no atypical cells. The peripheral blood OKT4/OKT8 ratio, measured using flow microfluorometry, was 3·1 (normal range 0·9–1·8). Haematoxylin and eosin stained biopsy specimens from the lesions showed a dense lymphoid cell infiltrate throughout the entire dermis with little tendency to epidermotropism (Fig. 2). Higher magnification revealed that the dermal infiltrating cells were mainly medium sized atypical lymphoid cells. Most of them had relatively pale staining vesicular nuclei with prominent nucleoli. Some mitotic figures were also present. Electron microscopic examination showed that the infiltrating atypical lymphoid cells had a relatively electron dense cytoplasm with numerous ribosomes, a few mitochondria, occasional dense bodies and short sections of rough endoplasmic reticulum. Each nucleus had a large concentration of heterochromatin and a prominent central nucleolus. Although most of the nuclei had an irregular surface, marked nuclear indentation was not observed (Fig. 3). The eruption began to regress. However, more than 10 new nodules subsequently appeared on the trunk. We suspected malignant lymphoma and carried out further investigations including immunohistochemical studies and DNA analysis in order to exclude other lymphoproliferative disorders. The patient was treated with local injections of β-interferon 1 000 000 units for each lesion twice daily for 10 days without effect. In April 1987, he died suddenly of acute renal failure.

METHODS

Three biopsy specimens were obtained from three different nodules on the trunk. Each specimen was divided into several portions for immunohistochemical studies and DNA analysis.

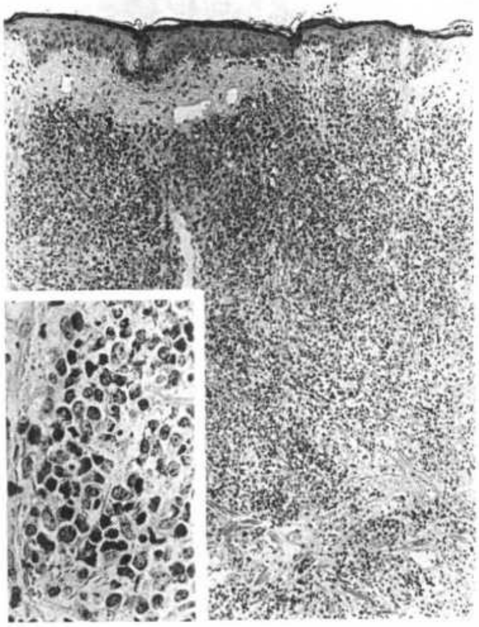

FIGURE 2. Dense atypical lymphoid cell infiltrate throughout the dermis with little tendency to epidermotropism (H&E; original × 50). Inset, higher magnification (original × 200).

Immunohistochemical studies

Each specimen was fixed in periodate-lysine-paraformaldehyde (PLP) medium, transferred through increasing concentrations of sucrose and frozen. Cryostat sections (6μm) were stained with the following monoclonal antibodies by an indirect immunoperoxidase method using an avidin-biotin-peroxidase complex: OKT8, OKT11 (Ortho Pharmaceutical Corp, Rariton NJ, U.S.A.); Leu-3a, anti-HLA-DR (Becton Dickinson FACS Systems, Sunnyvale, CA, U.S.A.) and anti-B1 (Coulter Electronics, Bethesda, MD U.S.A.).

DNA analysis

DNA was extracted and purified using standard procedures.[2] The DNA samples were digested with *Bam* HI and *Eco* RI restriction enzymes (Takara Shuzo Co, Kyoto, Japan). The digested DNA was fractionated by 0·8% agarose gel electrophoresis and transferred to nitrocellulose filters.[3,4] Filter-bound DNA fragments were then hybridized with a ^{32}P-labelled human T cell receptor β-chain gene probe and visualized by autoradiography.[5] The gene probe, kindly provided by Professor Honjo, Kyoto University was a *Hind* III-*Eco*R1 fragment of human genomic DNA encoding the Cβ1 region.[6] As this probe strongly cross hybridizes with the Cβ2 region, two constant regions of the T cell receptor β-chain gene (Cβ1 and Cβ2) are detectable using this probe alone.

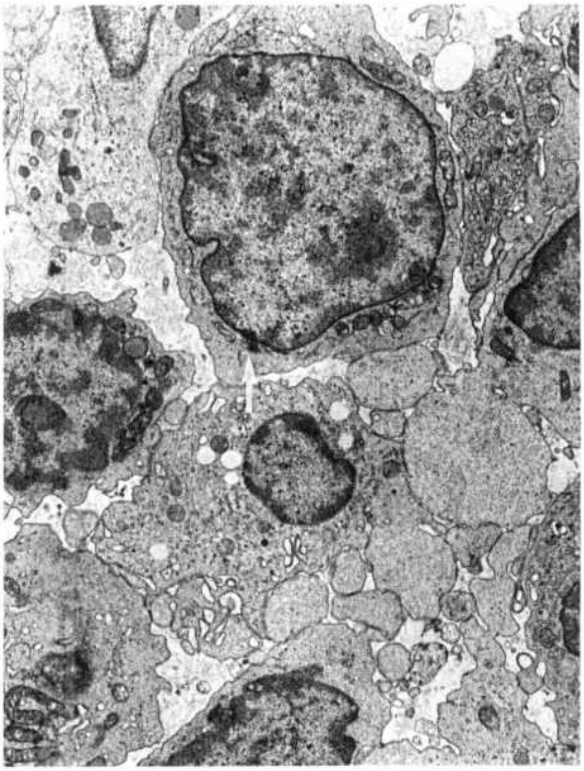

FIGURE 3. Infiltrating atypical lymphoid cells with an electron dense cytoplasm and an irregular nuclear surface. Arrow indicates a dense body. (Uranyl acetate and lead citrate; original × 7000).

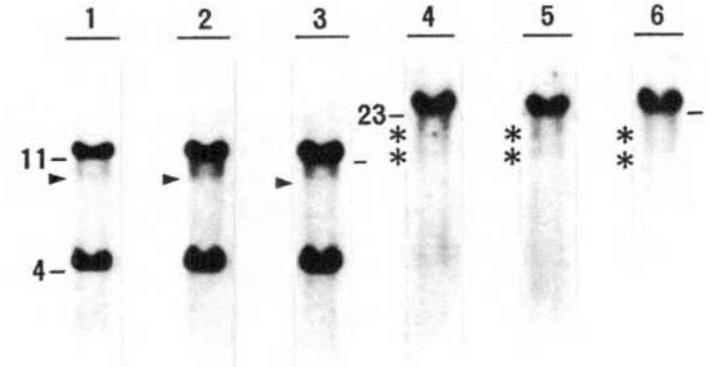

FIGURE 4. Autoradiographs of DNA analysis. Lanes 1 and 4 nodule 1, lanes 2 and 5 nodule 2, and lanes 3 and 6 nodule 3. Lanes 1–3 digests with *Eco*RI and lanes 4–6 digests with *Bam*HI. Dashes indicate unrearranged germ-line fragments, arrows and asterisks indicate the rearranged fragments. Numbers indicate mol wt in kb determined from internal standards.

RESULTS

Immunohistochemical studies

Lymphoid cells gave a positive reaction with monoclonal antibodies OKT11 (approximately 100% of the dermal infiltrate), OKT8 (95%) and Leu-3a (5%), but did not stain with anti-HLA-DR or anti-B1. These findings indicated that the lymphoid tumour cells were of a suppressor/cytotoxic T cell type.

DNA analysis

The results of the DNA analysis are shown in Figure 4. All three nodules revealed the same rearrangement pattern. The 9·0 kilobase (kb) band appeared in all three samples on digestion with *Eco*RI. The 14·5 kb and 12·0 kb bands appeared in all three samples on digestion with *Bam*HI. These results indicated that the three lesions contained cells from the same clone and, therefore, that the cells in all three lesions had originated from a single cell.

DISCUSSION

The infiltrating cells in our patient had a suppressor/cytotoxic T cell surface phenotype and showed atypical appearances on histological examination. However, benign lymphoproliferative disorders such as Jessner's lymphocytic infiltration also show a uniform surface phenotype[7] and under some conditions (for example, inflammatory reactions) benign lymphocytes can mimic atypical cells. However, the DNA analysis indicated that each nodule in our case had originated from the same single cell, this representing a monoclonal proliferation, as the three biopsy specimens revealed the same rearrangement pattern. Therefore, taking the immunohistochemical results into consideration, we suggest that our case is a suppressor/cytotoxic T cell lymphoma.

A diagnosis of a malignant lymphoma has been made in the past mainly on histology. However, the usefulness of DNA analysis in making an accurate diagnosis of leukaemias and lymphomas has received attention recently.[8] In dermatology this method was first applied to the diagnosis of mycosis fungoides and lymphomatoid papulosis.[9,10] Lymphomatoid papulosis, which should be differentiated from malignant lymphoma, also showed a clonal rearrangement pattern.[10] In the present case, clonal subsets from the three nodules examined revealed the same rearrangement pattern on DNA analysis, indicating this case to be a malignant lymphoma rather than lymphomatoid papulosis. It would be possible to make a diagnosis from a single specimen if the specific rearrangement pattern in malignant lymphoma were to be found. However, it is preferable to take several specimens from different lesions in order to make a more reliable diagnosis.

The clinical course in the present case resembled that of lymphomatoid papulosis with respect to spontaneous regression. Although malignant lymphomas showing spontaneous regression are relatively rare, several cases have been reported.[11] Macaulay[12] proposed the term 'rhythmic paradoxical eruptions' to describe eruptions with a rhythmic pattern of repeated evolution and involution in malignant lymphomas and lymphomatoid papulosis. This author also mentioned that cases of malignant lymphomas with this type of eruption tended to have a good prognosis in spite of histological evidence of malignancy. Our case appeared to belong to this category. In the present study, we took three biopsy specimens at the same time. However, in cases having rhythmic eruptions, it would be worthwhile to examine each phase of evolution for a possible change in the clonal subsets.

ACKNOWLEDGEMENTS

We thank Professor Honjo, Department of Medical Chemistry, Faculty of Medicine, Kyoto University and Professor Nakanishi, Institute for Immunology, Faculty of Medicine, Kyoto University, for providing the gene probes and for helpful advice.

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British Journal of Dermatology (1988) **118**, 267–301

Society Proceedings

British Society for Investigative Dermatology Annual Meeting, Cambridge, September 1987

PAPERS

Preferential binding of bullous pemphigoid sera to permeable cells in an ELISA. A.L.COOK, R.J.PYE AND T.H.P.HANAHOE,* *Dermatology Research Unit, Addenbrooke's Hospital, Cambridge, and *School of Natural Sciences, Hatfield Polytechnic, Hatfield, Herts, U.K.*

An enzyme-linked immunosorbent assay (ELISA), employing a human bladder squamous cell carcinoma line (SCaBER) as substrate to detect the autoantibodies in pemphigoid and pemphigus, has been described previously (Cook & Pye, *Br J Dermatol*, 1985; **113**: 766). However, there was no correlation between indirect immunofluorescent (IIF) titres on air-dried human skin sections and binding indices on intact, viable SCaBER cells by ELISA. This study examines whether the discrepancies were due to different levels of antigen expression or accessibility within these substrates.

Viable SCaBER cells were used in the ELISA and compared with 0·1% saponin permeabilized cells. Control sera from 14 individuals showed reduced binding to permeable cells. IIF-positive sera from 21 of 25 patients with bullous pemphigoid (BP) showed significantly increased binding to permeable cells ($P < 0{\cdot}0001$, Wilcoxon signed rank test). Two of 6 IIF-negative BP sera showed increased binding. In contrast, 9 IIF-positive pemphigus vulgaris (PV) sera, 4 IIF-positive pemphigus foliaceus (PF) sera and 3 IIF-negative PV and PF sera showed no significant difference between binding to intact or permeabilized cells.

These results suggest that the BP antigen(s) are both extra- and intracellular in SCaBER cells, but that PV antigen(s) are extracellular. Previous work by Westgate *et al.* (*J Invest Dermatol* 1985; **84**: 218) and Mutasim *et al.* (*J Invest Dermatol* 1985; **84**: 47) demonstrated that BP antigen(s) are localized to the cytoplasmic face of hemidesmosomes by immunoelectron microscopy in rat and mouse epidermal cells respectively.

Preferential activation of CD4 T-lymphocytes in the lamina propria of dermatitis herpetiformis and coeliac disease. C.E.M.GRIFFITHS,* I.BARRISON,* J.N.LEONARD, K.CAUN,† H.VALIDMARSSON‡ AND LIONEL FRY, **Departments of Dermatology and Gastroenterology, St Mary's Hospital, London, †Department of Gastroenterology, West Middlesex Hospital, Middlesex, U.K. ‡Department of Immunology, Lanspitalinn, Reykjavik, Iceland.*

The distribution and activation of T-lymphocyte subsets in the small intestinal mucosa of coeliac disease and dermatitis herpetiformis subjects on a normal diet has been studied and compared with normal controls. Double-labelling immunofluorescence techniques in conjunction with monoclonal antibodies were used on cryostat tissue sections.

In the epithelium there was no difference between controls, coeliac disease and dermatitis herpetiformis in that the majority (97%) of T cells present were of the CD8 subset, none of which were activated. Intestinal epithelial cells demonstrated staining for HLA-DR, the intensity being proportional to the degree of enteropathy.

The lamina propria demonstrated the major difference between the 3 groups, in coeliac disease and dermatitis herpetiformis there was a significant increase in CD3 cells compared with normals. An increase in the proportion of activated CD4 cells further differentiated the disease groups from normal. In normal controls the ratio of activated CD4 cells to activated CD8 cells was 1·24 whilst for dermatitis hepetiformis and coeliac disease it was 3·66 and 4·14, respectively.

As the major differences between the normal and the disease groups were in the lamina propria it appears that this is the primary site of activation in dermatitis herpetiformis and coeliac disease. It is postulated that prior sensitization to gliadin induced the observed changes and that the resultant enteropathy is a result of a delayed-type hypersensitivity reaction against gliadin at this site.

Characterization of the antigen in chronic bullous dermatosis of childhood and adult linear IgA disease.

F. WOJNAROWSKA*, P.WHITEHEAD†, B.BHOGAL‡, M.M.BLACK‡, AND I.M.LEIGH†, **The Slade Hospital, Oxford, †The London Hospital, and ‡Institute of Dermatology, London, U.K.*

Previous studies have suggested that the antigen in chronic bullous disease of childhood (CBDC) and adult linear IgA disease (LAD) are identical and differ in ultrastructural localization and tissue distribution from both the bullous pemphigoid (BP) and epidermolysis bullosa acquisita (EBA) antigens (Aboobaker *et al.*, *Br J Dermatol* 1987; **116:** 293; Pothupitiyia *et al.*, *Br J Dermatol* 1987; **116:** 443; Bhogal *et al.*, *Br J Dermatol*, in press).

Sera from 6 patients with CBDC and 3 patients with adult LAD were immunoblotted against both cultured keratinocyte supernatant and dermal extracts (8 M urea, 0·6 M 6–8 mercaptoethanol overnight at 4°C; Twobin *et al.*, *Proc Natl Acad Sci USA* 1979; **76:** 4350). The results were compared with immunoblots using sera from patients with BP and EBA antibodies (identified by immunofluorescence, split skin studies and immunoelectron microscopy), the murine monoclonal antibody to type VII collagen, LH 7·2, and normal human sera.

Three CBDC and 1 adult LAD sera gave positive results. All the positive sera identified an identical protein of molecular weight 285 kd, which was present only in the dermal extracts. The BP sera, as expected, gave negative results with the dermal extracts. The EBA sera and LH 7·2 monoclonal antibody blotted at 250 kd with the dermal extracts.

Thus, the antigens in CBDC and adult LAD are identical. The antigen is present in the dermis, confirming previous immunoelectron microscopy studies (Bhogal *et al.* 1987) at a similar site to the EBA/type VII collagen molecule, and is distinct from the BP antigen.

Ontogeny of a pemphigoid gestationis related antigen.

S.E.KELLY, B.BHOGAL*, F.T. WOJNAROWSKA AND M.M.BLACK*, *Departments of Dermatology, Slade Hospital, Oxford and *Institute of Dermatology, London, U.K.*

Pemphigoid gestations (PG) is a rare blistering condition of pregnancy which appears to be related to bullous pemphigoid (BP). The subepidermal bullae which characterize both diseases are thought to be caused by immune mediated tissue damage. Circulating anti-basement membrane zone (BMZ) antibodies may be detected in the serum of both groups of patients. In PG the antibody is avidly complement-fixing and the serum anti-BMZ complement-fixing agent is termed PG factor. The antigen against which PG factor is directed has not been characterized. The relationship between PG and pregnancy suggests that the antigen may be a molecule present in both placenta and skin. We studied the antigen-binding specificity of 25 PG sera using an indirect immunofluorescent technique with as substrates: skin; skin incubated in 1 M NaCl and chemically separated through the lamina lucida; full-term placenta; placenta from 22 weeks pregnancy; and placenta from 12 weeks pregnancy. In addition, 3 linear IgA disease (LAD) sera and 3 BP sera were studied in a similar way. The complement-fixing factor was observed to bind to the epidermal basal keratinocytes and to amnion of placenta and umbilical cord. This was seen in both full-term and 22 weeks gestation placenta, but not in the placenta from 12 weeks gestation. This pattern of antibody deposition identifies an antigen shared between skin and placenta, present in placenta from the second trimester of pregnancy onwards. This cross reactivity is not surprising as skin and amnion are both derived from the ectodermal germ layer during embryogenesis. LAD antigen and BP antigen were also identified from the second trimester onwards.

Direct immunofluorescence of suction blisters in bullous and cicatricial pemphigoid.

V.A.Venning, F.Wojnarowska and P.R.Millard*, *Department of Dermatology, Slade Hospital and *Department of Histopathology, John Radcliffe Hospital, Oxford, U.K.*

Skin separated by a suction blister technique is cleaved through the lamina lucida and it has been reported that the bullous pemphigoid (BP) antigen adheres to the roof of such a blister, while the antigen(s) of cicatricial pemphigoid (CP) are components of its floor (Woodley *et al.*, *J Invest Dermatol* 1983; **81:** 149; Fine *et al.*, *J Invest Dermatol* 1984; **82:** 36).

To test this hypothesis, direct immunofluorescence studies were performed on intact skin and on skin separated by a suction blister in 21 patients with BP and 11 patients with CP.

The mean blistering time was similar in both groups. Of the BP patients, 7 had positive direct immunofluorescence (IF) of intact skin. Of these, the distribution of positive IF in the blister was: negative in 3; roof only in 1; base only in 1; roof and base in 2. A further patient had negative IF in intact skin but positive in both roof and base of the blister. In CP, positive IF occurred in the roof only in 3 patients, and floor only in 2. Only 2 of these 5 patients had positive direct IF of intact skin.

The different patterns of blister IF could not be correlated with clinical differences between the patients. We conclude that the distribution of *in vivo* bound immunoreactants in suction blisters does not distinguish BP from CP. Either the antigen(s) concerned in both diseases are heterogeneous or the technique of separating skin by a suction blister does not consistently separate the various lamina lucida antigens into the same compartments.

Type VII collagen is the target for autoantibody binding in epidermolysis bullosa acquisita.

P.Whitehead*, R.A.Briggaman†, F.Wojnarowska‡, and I.M.Leigh*, **The London Hospital, London, U.K. ‡University of North Carolina, Chapel Hill, U.S.A. †The Slade Hospital, Oxford, U.K.*

Type VII collagen is a newly described basement membrane collagen which is the major component of the anchoring fibril (Sakai *et al.*, *J Cell Biol* 1986; **103:** 1577). The murine monoclonal antibody LH 7·2 reacts with the carboxy terminal region of type VII collagen and has been shown to bind on immunoelectron microscopy to the inferior border of the lamina densa (Leigh *et al.* unpublished). Thus, the carboxy terminal anchors the long α helical collagenous region of type VII collagen, i.e. the filamentous part of the anchoring fibril, to the lamina densa. Antigenic determinants of type VII collagen therefore span the lamina densa to sublamina densa zone.

The sera from 2 patients with epidermolysis bullosa acquisita (EBA), previously characterized by immunoelectron microscopy, immunofluorescence and immunoblotting were blotted against preparations of type VII collagen from cultured keratinocyte supernatant and dermal extracts (8 M urea 0·6 M 6–8 mercaptoethanol, overnight at 4°C (Bruckner-Tudeman *et al.*, *European J Biochem*, in press) in comparison with LH 7·2 antibody (Twobin, *Proc Natl Acad Sci USA* 1979; **76:** 4350). Positive results were obtained only with the dermal extracts.

EBA antisera showed binding to the same molecular weight peptides as LH 7·2 confirming that the target for EBA autoantibody is type VII collagen (Woodley *et al*, *J Invest Dermatol* 1987; **88:** 525).

Activated Harvey ras oncogene with a codon 12 mutation derived from a basal cell carcinoma.

S.I.White and A.Balmain*, *Department of Dermatology, University of Glasgow and *Beatson Institute for Cancer Research, Glasgow, U.K.*

The activated *ras* oncogene has been detected in human malignancies of many histological types. In all cases of activation a point mutation at either codon 12 or 61 has been found. Basal cell carcinoma (BCC) is the commonest skin malignancy and with ultraviolet light as a major aetiological factor in its genesis an activated oncogene might be expected.

High molecular weight DNA from 10 histologically proven BCCs was assayed for the presence of an activated oncogene by the calcium phosphate co-precipitation method of DNA transfection. Two BCCs proved positive. Only one of these proved positive in second and third round transfections. DNA from these transfections was used in the subsequent experiments.

Analysis of the DNA by Southern blotting revealed the original BCC to be heterozygous for the Harvey-

ras gene. First, second and third round transfection DNA digested with the endonuclease *Bam*H1 and probed with the human Harvey-*ras* probe (T24), showed the presence of the Harvey-*ras* oncogene but with an abnormal fragment length of 12·5 kb in the first and second round DNA and 8·0 kb in the third round DNA (expected 6·0 kb). Polyacrylamide gel electrophoresis and immunoprecipitation of the p21 protein produced by a cell line containing the BCC gene showed a similar pattern to the EJ DNA (the human Harvey-*ras* oncogene known to contain a codon 12 mutation). A codon 12 mutation would lead to loss of an MSP1 endonuclease site and instead of two fragments of 355 bp and 56 bp one fragment of 411 bp. MSP1 digestion of our BCC transfection DNA showed a fragment of 411 bp in all 3 rounds. A Northern blot confirmed the DNA message to be the same as EJ DNA.

The human Harvey-*ras* oncogene has many *Pst* I endonuclease sites along its length. Using 4 of these fragments as probes, the 5′ flanking sequence, exon 2 and intron 2 were amplified but of the expected fragment size. A probe encompassing the 4th exon and the 3′ flanking sequence showed an abnormally large fragment of over 2 kb compared with the expected 769 bp.

The transforming gene from a basal cell carcinoma has from the 5′ flanking sequence to the second intron homologous to human Harvey-*ras*, there is a codon 12 mutation and rearrangement towards the 3′ end of the gene.

Skin cancers and their relationship to human papilloma virus infection in a group of renal allograft recipients. E.C.BENTON, M.H.BUNNEY, B.B.BARR*, K.MCLAREN†, K.BLESSING†, I.W. SMITH* AND J.A.A.HUNTER, *University Departments of Dermatology, *Virology and †Pathology, Royal Infirmary, Edinburgh.*

Renal allograft recipients (RAR), especially those who have had their grafts for over 10 years, commonly develop viral warts, keratoses, small scaly plaques and skin cancers. Their immunocompromised state and this array of cutaneous lesions resembles the situation in epidermodysplasia verruciformis (EV), where skin cancers are associated with unusual types of human papilloma virus (HPV) infection, including HPV5.

We have demonstrated by DNA blot hybridization, the presence of HPV5 DNA in 30 lesions from a group of 10 RAR including 5 patients with single or multiple skin cancers and 4 patents with EV-like plaques. Ten of 14 squamous cell carcinomata were positive for HPV5 DNA.

Histologically, symmetry of lesions, papilliferous architecture, patterns of vascularity, and cytological features seen in many in situ and squamous cell carcinomas were suggestive of a viral contribution to the aetiology. These features became less prominent but were, nevertheless, still present as the degree of dysplasia increased.

The findings suggest that HPV5 is a factor in carcinogenesis in renal allograft recipients.

An immunohistochemical study of basement membrane components in cutaneous intra-epithelial and invasive squamous cell carcinoma. M.J.TIDMAN, R. CERIO*, I.M.LEIGH† AND D.M.MACDONALD, *Laboratory of Applied Dermatopathology, U.M.D.S. (Guy's Campus), *Institute of Dermatology U.M.D.S., †Experimental Dermatology Research Laboratory, London Hospital, London, U.K.*

The mechanisms whereby malignant epithelial cells breach the basement membrane (BM) in the course of local invasion and metastatic spread are poorly understood. In this study, BM constituents were demonstrated on frozen cutaneous tissue from 5 intra-epithelial and 9 invasive squamous cell carcinomata (SCC) and from 6 keratoacanthomata by an indirect immunoperoxidase technique, employing antibodies to components of the lamina lucida (anti-laminin, GB3), lamina densa (anti-type IV collagen) and anchoring fibrils (anti-type VII collagen).

The BM changes beneath intra-epithelial SCC varied from mild reduplication of the immunoreactants to a moderate degree of attenuation and reduplication, with occasional focal breaks in continuity. Similar changes were evident beneath keratoacanthomata. BM was present around the tumour masses of infiltrating SCC, which included a cutaneous metastasis from a vulval SCC, although attenuation and focal deficiencies were more prominent. Furthermore, there was a consistent disproportionate diminution of type VII collagen immunoreactivity around invasive SCC, compared with the other BM components.

However, no abnormality of immunoreactive laminin and type IV collagen was apparent around dermal vascular channels, including those in close proximity to islands of malignant tumour cells almost devoid of BM.

In conclusion, persistence of a partially disrupted epithelial BM is a feature of stromal invasion, and possibly metastasis, by SCC. The normality of vascular BM in close juxtaposition to SCC argues against an indiscriminate release of degrading enzymes into the stroma by tumour cells, and is consistent with diminished capacity for the synthesis of BM material by malignant keratinocytes.

Initial experience with use of an anti-melanoma monoclonal antibody in imaging metastatic melanoma. V.R.DOHERTY, A.T.ELLIOTT, T.MURRAY, F.G.ADAMS AND R.M.MACKIE, *Departments of Dermatology, Clinical Physics, Pharmacy and Radiology, Western Infirmary, Glasgow, U.K.*

Monoclonal antibodies (MCA) for diagnostic imaging have been introduced recently, with the prospect of advancing to therapeutic use if successful and safe. Initial diagnostic studies in patients using MCAs have shown diverse results, particularly in cases of epithelial tumours. Some of the most encouraging results have been obtained using monoclonal antibodies to malignant melanoma. We have used an MCA for radioimaging in 15 patients with malignant melanoma and compared results obtained with conventional CT scanning.

The MCA used was murine, of the IgG2a subclass with a molecular weight of 150 000 daltons. We compared its use as a radioimaging substance with conventional staging procedures, in particular with CT scanning. The MCA was linked with indium I^{111} and screening carried out at 24 and 96 h post injection. Where feasible, additional studies at 4, 48 and 72 h were carried out in addition to emission tomography at 96 h. There was evidence of uptake of MCA into tumour sites as early as 4 h, with no subsequent clearance from tumour sites. Non-tumour sites, notably the liver and spleen, exhibited slow biological clearance. Uptake into tumour sites exceeded 1%, which would be adequate for targeted therapy. Due to the high uptake in the liver and spleen, imaging of possible metastases in these sites was difficult but was aided by a concomitant conventional isotope scan.

In 6 of the 15 patients studied there was complete concordance in findings of the CT scan and MCA scanning. In 5 patients the MCA scan demonstrated unrecognized sites of disease, as it gives an image of the entire body unlike conventional CT scans. In 4 cases the CT scan was able to detect metastases in sites reported as normal on monoclonal antibody scanning (lung and intra-abdominal lymph nodes).

This early study has shown that MCA scanning is a safe and well tolerated out-patient procedure. The results for radioimaging compare favourably with existing staging methods in malignant melanoma, in particular with CT scanning.

$1\alpha 25$ dihydroxy vitamin D_3 ($(OH)_2D_3$) increases intracellular free calcium in B16 melanoma. S.HILL*, S.S.BLEEHEN† AND S.MAC NEIL*, **Department of Medicine, Clinical Sciences Centre, Northern General Hospital, Sheffield and †Department of Dermatology, Royal Hallamshire Hospital, Sheffield, U.K.*

Several groups have recently addressed the question of whether the active metabolite of vitamin D_3, ($(OH)_2D_3$) does or does not contribute to pigment production induced by UVB irradiation in cultured melanocytes and melanoma cell lines. To investigate this question in cultured B16 murine melanoma we looked for evidence that these cells were capable of recognizing and responding to $(OH)_2D_3$. No significant effects of vitamin D_3 or $(OH)_2D_3$ were found on the two major intracellular signalling systems—cyclic AMP and phosphatidyl-inositol (PI) turnover.

However, using the fluorescent calcium chelator Fura-2 (4 μM), $(OH)_2D_3$ was found to increase intracellular calcium in subconfluent monolayers of B16 melanoma. This effect occurred at 10^{-10} to 10^{-9} M, well within the physiological range of action of $(OH)_2D_3$ and was not found with vitamin D_3 itself. The response was as great or greater than the response to 10 μM A23187 (a potent calcium ionophore), was apparent within 15 min of addition of $(OH)_2D_3$, and persisted for 10–120 min before the cells reattained basal levels of intracellar free calcium. The observed increase in calcium was less likely to be seen in fully confluent cells, indicating possible changes in membrane characteristics associated with the state of proliferation of the cell.

The increase in intracellular calcium was entirely dependent on the presence of extracellular calcium. This is consistent with $(OH)_2D_3$ acting independently of PI hydrolysis (which results in an elevation of intracellular calcium from intracellular stores).

Our data demonstrating that B16 melanoma cells can respond to physiologically relevant concentrations of $(OH)_2D_3$ with a marked and sustained elevation of intracellular free calcium increases our understanding of the mechanism of action of $(OH)_2D_3$ and may be relevant to how $(OH)_2D_3$ is reported to contribute to the melanogenic response to UV.

Changes in endothelial cell mass, luminal volume and capillary number in the gravitational syndrome. M.M.Mourad, S.P.Barton and R.Marks, *Department of Medicine, University of Wales College of Medicine, Heath Park, Cardiff, U.K.*

Healing in the gravitational syndrome does not necessarily accompany the extensive neoangiogenesis of the ulcerated site. This paradox suggests that either vascular function of the new capillaries is inadequate or that intra-tissue perfusion of O_2 is inadequate for other reasons. To characterize the vascular alterations in the gravitational syndrome we studied biopsies from 31 patients (12 male, 19 female, mean age 69 ± 12 years) and 10 normal control subjects (3 male, 7 female, mean age 47 ± 6 years) taken from the ulcer edge and the middle of the lower leg, respectively. Biopsies were assessed for vascular endothelial mass (VEM), luminal volume (VLV) and number of capillaries per unit tissue volume (NV) using computer assisted planimetry. Eight predetermined fields in each of 6 predetermined histological sections were analysed from each biopsy. Endothelium was traced around its outer and inner margins using a camera lucida/digitiser cursor. The results are shown below:

		Mean*	median*	SD*	P
VEM	Patient	51·5	50·0	21·9	0·001
	Control	23·6	23·0	5·3	
VLV	Patient	5·9	5·1	3·9	0·05
	Control	3·0	3·0	1·1	
NV	Patient	2·29	2·0	0·9	0·01
	Control	1·32	1·42	0·3	

* × 1,000

Other analyses indicated that VEM, VLV and NV are positively related to the number of inflammatory cells and that VEM is positively related to epidermal thickness. The lack of a simple correlation of these parameters with age and the marked difference between controls and patients suggest that these differences are little influenced by age. These parameters have also been correlated with a clinical index of disease activity and strongly suggest that there is a negative relationship between VLV and disease activity. Overall, the data suggest that the prime determinant of the clinical outcome is the patency and adequacy of the vascular lumina.

Reduced threshold for collagen-induced platelet aggregation in whole blood from patients with Raynaud's phenomenon. M.J.D.Goodfield, M.A.Orchard* and N.R.Rowell, *Departments of Dermatology and *Medicine, The General Infirmary at Leeds, Leeds, U.K.*

Raynaud's phenomenon (RP), characterized by episodic attacks of pallor, cyanosis and reactive hyperaemia, is a vascular symptom of unknown aetiology. In systemic sclerosis (SS), the vascular lesions are accompanied by cutaneous sclerosis, and previous reports have suggested that platelet aggregation is abnormal and could contribute to the pathogenesis. (Kahaleh *et al.*, *Ann Intern Med* 1982; **96:** 610). Differences in platelet responsiveness between primary and secondary RP might provide further evidence of an aetiological role. Platelet aggregation in whole blood was investigated in 15 patients with systemic sclerosis, 5 patients with primary RP, and 15 age and sex-matched controls. Subjects rested for 30 min before blood samples were taken without stasis, via a 19G needle into 3·8% trisodium citrate anticoagulant. 0·5 ml of blood was transferred to cuvettes, and equilibrated at 22°C for 30 min, before being stirred at 750 r.p.m. at 37°C. Aggregation was induced by collagen (0·01–1·0 μg/l), adrenalin (0·1, 0·5 and 1·0 μm/l) and ADP (0·1, 0·25 and 2·5 μm/l). Aliquots for counting free platelets were taken and assessed using an Ultraflow 100 particle counter.

Platelet aggregation was significantly increased in patients with RP, both primary and secondary, at all doses of collagen ($P < 0{\cdot}01$), and the mean dose giving 50% aggregation (EC50) was significantly lower in the SS group (0·04 ± 0·06 μg/l), and the RP group (0·07 ± 0·05 μg/l) than in the controls (0·23 ± 0·06 μg/l) ($P < 0{\cdot}01$). The difference in EC50 between SS and RP was significant ($P < 0{\cdot}05$). Aggregation was not significantly different with the other aggregating agents.

Increased sensitivity of platelets to aggregation by collagen in whole blood may be of importance in the pathogenesis of Raynaud's phenomenon. The further increase in sensitivity in SS may be important in the development of cutaneous sclerosis.

Modulation of endothelial cell procoagulant activity and prostacyclin release by sera from patients with a lupus anticoagulant in association with systemic lupus erythematosus. M.H.A.Rustin, Helen A.Bull, S.J.Machin*, D.A.Isenberg†, M.L.Snaith† and Pauline M.Dowd, *Departments of Dermatology, *Haematology and †Rheumatology, The Middlesex Hospital, Mortimer Street, London, U.K.*

The cause of the thrombotic tendency in patients having the lupus anticoagulant (LA) is unknown. Disturbed endothelial cell (EC) function interfering with the release of prostacyclin (PGI_2) and production of the procoagulant tissue factor may be pathogenetic factors. Therefore, the effect of sera from 9 normal subjects (NS), 9 with systemic lupus erythematosus (SLE) with LA and 13 with SLE without LA on the release of PGI_2 and production of procoagulant activity by cultured human EC was studied.

Confluent primary cultures of human umbilical vein EC were incubated with 1–20% sera for 24 h. After stimulation with the calcium ionophore A23187, 6-keto-$PGF_{1\alpha}$ (the stable metabolite of PGI_2) in the supernatant was measured. EC procoagulant activity was determined after incubation with 20% sera for 6 h.

A dose-dependent inhibition of 6-keto-$PGF_{1\alpha}$ was observed with all the patients' and control sera, but only the 10 and 20% sera from patients with SLE without LA produced a significantly greater inhibition than control sera ($P < 0{\cdot}05$ and $P < 0{\cdot}02$, respectively). Sera from patients with LA caused a significant increase in procoagulant activity ($P < 0{\cdot}001$) as measured by shortening of the two-stage partial thromboplastin time (estimation of intrinsic pathway activity) (NS 65·9 ± 3·4 s, SLE + LA 30·9 ± 1·6 s, SLE without LA 34·4 ± 1·5 s) whereas with the one-stage prothrombin time (estimation of extrinsic pathway activity) there was a significant shortening ($P < 0{\cdot}001$) with the LA group compared with SLE without LA (NS 37 ± 2 s, SLE + LA 31 ± 1 s, SLE without LA 39 ± 1 s) but neither were significantly different from the normal subjects.

The significant increase in procoagulant activity by sera from patients with SLE + LA may be a factor responsible for the thrombotic tendency in these patients.

Eicosanoids and lymphocyte chemotaxis. K.B.Bacon, R.D.R.Camp and F.M.Cunningham, *Institute of Dermatology, St. Thomas' Hospital, London, U.K.*

12(R)-hydroxy-5,8,10,14-eicosatetraenoic acid [12(R)-HETE] and leukotriene B_4 [LTB_4] are found in μg/g and ng/g quantities, respectively, in lesional psoriatic scale (Brain *et al*, *Br J Pharmacol*, 1984; **83:** 313; Cunningham *et al.*, *Prostaglandins* 1985; **30:** 497; Woollard, *Biochem Biophys Res Commun* 1986; **136:** 169). Since lymphocytic infiltration occurs in psoriatic lesions, human lymphocyte chemotactic responses to

12(R)-HETE, its epimer 12(S)-HETE and LTB$_4$ were measured. Responses to the standard chemoattractants zymosan activated plasma (ZAP), casein and N-formyl-methionyl-leucyl-phenylalanine (fMLP), were also obtained.

Mixed human peripheral blood lymphocytes were prepared by Ficoll-Hypaque density gradient centrifugation, monocytes being removed by adherence to plastic for 1 h, followed by culture overnight, to yield a lymphocyte population of 99·5±0·4% purity. Chemotaxis was assessed by use of a 48-well microchemotaxis chamber and 8 μm pore size polyvinylpyrrolidone-free polycarbonate filters (Harvath *et al., J Immunol Methods* 1980; 37: 39). Responses were quantified by image analysis and expressed as migration indices [area of lower surface of filter occupied by cells in response to chemoattractant (mm^2)/area occupied by randomly migrating cells (mm^2)].

Dose-related chemotactic responses were obtained to 12(R)-HETE and LTB$_4$, maximum migration indices being 2·99±0·35 at 10^{-5} M and 2·96±0·41 at 10^{-7} M, respectively ($n=4$). 12(S)-HETE, in contrast to 12(R)-HETE, was a weak chemoattractant, maximum migration indices being 1·37±0·15 and 2·72±0·36 for 12(S)-HETE and 12(R)-HETE, respectively, at 10^{-5} M ($n=5$). Dose-related chemotaxis was also obtained in response to ZAP, casein and fMLP, maximum migration indices being 3·38±0·35 for 5% ZAP ($n=8$), 1·82±0·11 for 1 mg/ml casein ($n=5$) and 1·94±0·27 for 10^{-7} M fMLP ($n=4$).

Although LTB$_4$ is at least 200 times more potent than 12(R)-HETE as a lymphocyte chemoattractant *in vitro*, 12(R)-HETE is found in 1000-fold greater amounts in extracts of lesional psoriatic skin and may therefore be of equal importance to LTB$_4$ in eliciting the lesional lymphocyte infiltrates.

Nickel sensitivity and its detection by the *in vitro* stimulation of lymphocytes by nickel. K.M.Everness, D.J.Gawkrodger, P.A.Botham* and J.A.A.Hunter, *University Department of Dermatology, The Royal Infirmary, Edinburgh and *Central Toxicology Laboratory, Imperial Chemical Industries plc., Alderley Park, Cheshire, U.K.*

The lymphocyte transformation test (LTT) using 5 μg/ml nickel sulphate (NiSO$_4$) with peripheral blood mononuclear cells (PBM) separated on lymphopaque can be used to discriminate between nickel-sensitive individuals and controls. Thirty-nine NiSO$_4$ patch test positive patients and 25 controls were studied using 0–20 μg/ml NiSO$_4$ in 6 and 7 day assays. Thirty-four patients responded to 5 μg/ml NiSO$_4$ in the 6-day assay with stimulation indices (SI) $\geqslant 3$; 38 responded in the 7 day assay. None of the 25 controls reacted. SIs obtained from 6 patients before and 5 days after patch testing were not significantly different.

A suspension of enriched T cells was obtained from PBM by the passage of plastic-non-adherent cells down a nylon-wool column followed by glass adherence, Addition of 10^5 irradiated cells to 10^6 T cells reconstituted the nickel-specific response in 9 of 12 patients; the mitogenic (Con A) response was also reconstituted in cells from the same 9 nickel sensitive patients and all 6 negative controls. The values of SI obtained using PBM, and T cells reconstituted with adherent cells, were not significantly different.

Preliminary experiments have also been carried out in which responses to Con A and NiSO$_4$ have been reconstituted by the addition of $1–4\times10^{-5}$ irradiated epidermal cells to 10^6 T cells. The system is currently being used to investigate the role of different MHC Class II molecules in nickel-specific and Con A-induced T cell proliferation.

Comparative emigration of CD4 and CD8 lymphocytes into the site of a type IV hypersensitivity (tuberculin) skin reaction. Susan M.Morely*, J.S.Beck†, G.Lowe*, R.D.Brown‡, *Departments of *Dermatology, †Pathology and ‡Mathematics, University of Dundee, U.K.*

In a tuberculin reaction, most of the T lymphocytes (subsets CD4 and CD8) emigrating from the blood stream are found around blood vessels and adnexal structures; a minority migrate further into the dermis. This study was undertaken in patients with different diseases and controls, to see if regional variation in CD4:CD8 ratios occurs during emigration.

One hundred and four biopsies of skin tests to New tuberculin antigen, from 77 Indonesian subjects (44 with tuberculosis, 4 with leprosy and 29 controls) and 27 Scottish patients (18 with steroid-treated chronic lung disease and 9 haemophiliacs) were analysed using immunocytochemical stains and morphometric techniques as described previously (Gibbs *et al., J Clin Pathol* 1984; 37: 1227). Ratios of CD4 and CD8 densities were estimated in the perivascular foci and intervening dermis and compared with CD4:CD8 ratios of peripheral blood lymphocytes (estimated using FACS analysis for Scottish patients and fluorescent microscopic techniques for the Indonesian subjects).

In all subject groups, the CD4:CD8 ratio of peripheral blood (mean 1·77 ± 0·49) and perivascular foci (mean 1·81 ± 1·08), was similar. It was raised consistently in the intervening dermis, increasing in deeper levels (mean 3·26 ± 2·75, level 1, 5·72 ± 6·35, level 3). The differences in ratios between blood and intervening dermis were statistically significant ($P < 0·001$) and mainly due to reduction in CD8 cells.

The data suggests that emigration of lymphocyte subsets from the blood stream to the perivascular space occurs without bias; selective emigration occurs between the perivascular foci and adjacent dermis.

Effect of topical corticosteroid therapy on Langerhans cell function in human skin.

J.ASHWORTH, J.BOOKER AND S.M.BREATHNACH, *Department of Medicine (Dermatology), Charing Cross and Westminster Medical School, London, U.K.*

Topical corticosteroids are known to suppress both induction and elicitation phases of contact sensitivity in man, and are used to treat a wide variety of inflammatory skin disorders. The mechanisms by which they modulate cutaneous immune reactions are, however, incompletely understood. We have therefore investigated the immunomodulatory action of topical corticosteroids by studying and comparing their effects on (a) Langerhans cell (LC) expression of HLA-DR and T6/CD1a antigens and (b) LC antigen presenting function.

Volunteers applied Eumovate (clobetasone butyrate 0·05%), Betnovate (betamethasone valerate 0·1%) or Dermovate (clobetasol propionate 0·05%) ointment to one forearm, and inactive control vehicle to the contralateral forearm, twice daily for 7 days. Epidermal sheets were then harvested by the suction blister method. HLA-DR/T6 positive LCs were enumerated by a double immunofluorescence technique using fluoresceinated OKT6 and phycoerythrin-conjugated anti-HLA-DR. LC antigen presenting capacity was determined in the allogeneic epidermal cell–lymphocyte reaction (ELR), in which epidermal cells (EC) were cultured with allogeneic peripheral blood mononuclear cells, and in an autologous ELR, in which EC were cultured with purified autologous T cells with added concanavalin A (ConA) or tetanus toxoid. LC-dependent stimulation of T lymphocyte proliferation was measured by tritiated thymidine uptake.

Steroid therapy decreased the number of HLA-DR/T6 positive LCs per mm^2, expressed as a percentage of the controls (mean ± SEM), as follows: Eumovate 69·2 ± 8·8% ($n = 7$); Betnovate 66·1 ± 5·4% ($n = 12$); Dermovate 39·1 ± 3% ($n = 20$). The antigen-presenting function of LCs in the allogeneic ELR, expressed as a percentage of the controls (mean ± SEM), was similarly reduced: Eumovate 45·1% ± 10·7% ($n = 8$); Betnovate 41·9% ± 12% ($n = 7$); Dermovate 20·6% ± 5·6% ($n = 16$). Dermovate therapy reduced the presentation of ConA and tetanus toxoid in the autologous ELR to a mean of 21·5% and 19% of the controls, respectively ($n = 2$). Depression of LC function was not the result of a steroid-induced decrease in epidermal cell-derived thymocyte activating factor (ETAF)/interleukin 1 (IL-1), or increase in prostaglandin E_2 production by keratinocytes, since it could not be reversed by exogenous IL-1 or indomethacin. Co-culture of steroid-treated EC with control EC in the allogeneic ELR resulted in reduced T cell proliferation compared with control EC alone, suggesting the existence of some other inhibitory mechanism. Our results therefore clearly demonstrate that the anti-inflammatory effect of topical corticosteroids in man involves not only a reduction in the number of HLA-DR/T6 positive LCs, but also a marked, potency-related, decrease in the capacity of epidermal cells to present antigen to lymphocytes.

Cyclic adenosine monophosphate-phosphodiesterase activity in cultured keratinocytes from subjects with atopic eczema.

S.WRIGHT, P.WHITEHEAD, H.NAVSARIA, P.PURKIS AND I.M.LEIGH, *Department of Experimental Dermatology, The London Hospital, London, U.K.*

Increased cAMP-PDE activity in peripheral blood mononuclear leucocytes from patients with atopic eczema has been proposed as a biochemical marker for atopy. The lack of availability of pure preparations of epidermal cells has hindered attempts to assess cAMP-PDE activity in the epidermis of atopic subjects.

Using keratinocytes cultured from the skin of informed volunteers with atopic eczema by the methods of Rheinwald & Green (*Cell* 1975; **6**: 331) we have measured PDE activity by quantitating the production of

[^{3}H]adenosine from [^{3}H]cAMP by an epidermal cell homogenate and the action of an auxiliary enzyme, 5′ nucleotidase (Thompson & Appleman, *Biochem* 1971; **10:** 311).

Mean cAMP-PDE activity ± SEM for atopic keratinocytes was 6·86 ± 1·25 nmol/min/μg of protein ($n = 6$). Enzyme activity in control keratinocytes was 6·95 ± 1·95 nmol/min/μg of protein ($n = 4$). There was no correlation between the enzyme activity and the clinical severity of the eczema.

The fact that we have been unable to find any abnormality of cAMP-PDE activity in pure preparations of proliferating keratinocytes from subjects with atopic eczema suggests that this abnormality is likely to be restricted to certain cell populations.

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The effect of therapy on cyclic AMP phosphodiesterase activity in atopic dermatitis.

C.A.Holden and C.T.Yuen, *Skin Laboratory, St. Helier Hospital, Wrythe Lane, Carshalton, Surrey, U.K.*

Elevated cyclic AMP phosphodiesterase (PDE) activity has been proposed as the inherited biochemical basis for the hypersensitivity of atopic dermatitis (AD). We wished to determine the effect of therapy on the PDE activity of mononuclear leukocytes (MNL) from patients with AD.

MNL were prepared by Ficoll-Hypaque centrifugation of peripheral venous blood drawn from normal control subjects or patients with AD. All subjects avoided methylxanthine and caffeine-containing products for 14 h prior to venepuncture. The AD patients recieved topical emollients only for one week prior to venepuncture; subsequently they applied a fluorinated topical steroid ointment twice daily to all inflamed areas for a week and a further blood sample was taken. PDE activity of MNL was measured by radiometric enzyme assay using 0·025 μM [^{3}H]-cAMP as substrate. Kinetic studies employed concentrations from 0·025 μM to 2 μM cAMP. Results are expressed as pmol cAMP hydrolysed/min/mg protein ± SEM.

Untreated AD patients had significantly elevated MNL PDE activity (9·22 ± 0·46; $n = 13$) compared with normals (6·8 ± 0·42; $n = 11$) ($P < 0{\cdot}0025$). There was no difference in enzyme activity between patients with mild disease (9·23 ± 0·8; $n = 7$) compared with patients with extensive disease (9·19 ± 0·41; $n = 6$). Kinetic data showed three enzyme forms in normal MNL ($n = 5$) but only two enzyme forms in AD MNL ($n = 5$). Following treatment, patients showed a reduction of their PDE activity from 8·78 ± 0·58 to 7·6 ± 0·48 ($n = 8$) but this was not statistically significant. However, kinetic studies showed the re-emergence of a normal pattern of enzymes in MNL from all treated patients.

Our data confirm the presence of elevated PDE activity in MNL from AD patients with absence of one enzyme form in AD MNL. However, it would appear that the enzyme pattern can be returned to normal by one week's topical steroid treatment, with some reduction of total PDE activity.

Stimulation of phospholipase A2 in epidermal extracts by a phosphatase inhibitor.

P.H.Cartwright*, A.Ilchyshyn†, E.Ilderton* and H.J.Yardley‡, **Department of Dermatology, North Staffordshire Hospital Centre, Stoke-on-Trent, †Department of Dermatology, Coventry and Warwickshire Hospital, Coventry and ‡Department of Biological Sciences, University of Keele, U.K.*

Phospholipase A2 is present in normal epidermis and its activity is raised in psoriasis (Forster *et al*, *Br J Dermatol* 1985; **112:** 135). Phospholipase A2 activity is controlled by the protein lipocortin, the inhibitory activity of which is determined by its degree of phosphorylation: the more it is phosphorylated, the less it inhibits (for review, see Flower, *Nature* 1986; **320:** 20). Since a phosphotyrosyl protein phosphatase is present in epidermis (Friedman *et al. J Invest Dermatol* 1987; **88:** 489) it is instructive to include a phosphatase inhibitor, such as sodium pyrophosphate, in the homogenizing medium before measuring phospholipase A2 activity.

Normal and lesion-free psoriatic epidermis was homogenized in the presence or absence of sodium pyrophosphate at a final concentration of 30 mM (Resink *et al.*, *Eur J Biochem* 1983; **133:** 455). Phospholipase A2 activity was measured in these extracts as described.

For normal epidermis, the mean phospholipase A2 activity ± SEM in extracts prepared in the absence of pyrophosphate was 44·6 ± 3·9 nmol/mg protein/h ($n = 9$). For extracts prepared in the presence of pyrophosphate, the value was 168·6 ± 19·7 nmol/mg protein/h ($n=9$). This difference was significant ($P<0·001$; paired t-test). For lesion-free psoriatic epidermis, corresponding values were 91·5 ± 8·5 nmol/mg protein/h ($n=13$) and 170·5 ± 12·0 nmol/mg protein/h ($n=13$). This difference was also significant ($P<0·001$; paired t-test).

Thus, extracts of lesion-free psoriatic epidermis prepared in the absence of added pyrophosphate exhibited higher phospholipase A2 activities than similar extracts of normal epidermis ($P<0·001$; t-test), but this difference disappeared when 30 mM pyrophosphate was included in the homogenizing medium ($P>0·9$; t-test). These results suggest that phospholipase A2 is raised in psoriatic epidermis as a consequence of a defective protein phosphatase.

Proinflammatory effects of 12(R)-hydroxy-5,8,10,14-eicosatetraenoic acid in human skin. P.M.Woollard, G.M.Murphy, F.M.Cunningham, R.D.R.Camp and M.W.Greaves, *Institute of Dermatology, St. Thomas' Hospital, Lambeth Palace Road, London, U.K.*

12(R)-hydroxy-5,8,10,14-eicosatetraenoic acid [12(R)-HETE], the major stereoisomer of 12-HETE in lesional psoriatic scale, present in μg amounts, is more potent as a chemoattractant for human polymorphonuclear leucocytes (PMNs) *in vitro* than platelet-derived 12(S)-HETE (Cunningham *et al.*, *Br J Pharmacol* 1986; **87**: 107p; Woollard, *Biochem Biophys Res Commun* 1986; **136**: 169). The proinflammatory effects of 12(R)- and 12(S)-HETE have now been compared *in vivo* in human skin.

12(R)- and 12(S)-HETE (0·5–20 μg/site) were applied topically in a randomized fashion to the forearm skin of 5 healthy volunteers as previously described (Cunningham *et al*, *Prostaglandins* 1985; **30**: 497). 12(R)- and 12(S)-HETE (5 μg each) were also applied to two sites on the opposite limb. At 6 and 24 h the areas of erythema were recorded and changes in blood flow measured using a laser Doppler flowmeter. Biopsies were taken at 24 h from the two 5 μg application sites.

While both enantiomers caused dose-related erythema and blood flow changes at 6 and 24 h, differences were only seen at 24 h, where mean blood flow changes in response to 5 and 20 μg 12(R)-HETE were greater, although the difference was not statistically significant. Biopsies revealed PMNs in the epidermis, often in microabcesses, and in the dermis 24 h after application of 12(R)-HETE (25·2 ± 13·3 and 13·2 ± 5·1 cells/hpf; mean ± SEM; $n=5$). In contrast, 12(S)-HETE was not a chemoattractant under these conditions as there were 0·02 ± 0·02 and 1·0 ± 0·7 PMNs/hpf in the epidermis and dermis, respectively. There was no significant difference in the dermal mononuclear cells counts; 26 ± 9·9 and 20·5 ± 5·4 cells/hpf for 12(R)- and 12(S)-HETE, respectively.

Thus, 12(R)-HETE is a chemoattractant for human PMNs *in vivo* and appears to be much more potent than the lipoxygenase product, 12(S)-HETE. These results demonstrate the potential importance of 12(R)-HETE as a mediator of PMN infiltration in human skin.

Leukotriene B_4 (LTB_4) photodegradation by ultraviolet irradiation (UVB): a possible mechanism for the action of UVB phototherapy in psoriasis. B.Miller*, J. Ferguson, Catherine Green, T.MacLeod* and E.Raffle, *Departments of Dermatology and *Pharmacy, Ninewells Hospital, Dundee, Scotland.*

An early histological feature characteristic of lesional psoriasis is the transepidermal migration of polymorphonuclear leukocytes (PMN). Amongst PMN chemoattractants present in abnormally high concentrations in the stratum corneum of lesional psoriasis is leukotriene B_4 (LTB_4). Ultraviolet (UVB, 280–320 nm) phototherapy is an effective therapy for psoriasis. Longwave ultraviolet (UVA, 320–400 nm) alone has no such effect unless given at impractically high doses. We have studied UVB and UVA irradiation effects on LTB_4 using (i) high pressure liquid chromatography (HPLC), (ii) PMN under agarose chemotaxis assay, (iii) *in vivo* application on forearm skin with clinical grading and histological examination at 24 h. 100 μl (5 mg/ml) samples of LTB_4 were irradiated with UVB Phillips Colour 12 (290–320 nm) at dosages 100, 200, 500, 1000, 1500 mJ/cm² and with UVA, Sylvania PUVA (320–400 nm) at dosages ranging from 5 to 40 J/cm². HPLC demonstrated that LTB_4 was photodegraded by all dosages of UVB, 31, 40, 54, 64, 73% degradation. The UVA irradiation produced no such effect. In 10 healthy controls the PMN chemotactic differential (directional migration minus random migration) to unirradiated LTB_4 was 52·6 ± 11·6 μm, UVB irradiated 1500 mJ/cm² LTB_4 demonstrated a reduction at 29·8 ± 9·7 μm ($P < 0{\cdot}001$). As with the HPLC assay, UVA 40 J/cm² had no such effect. Control and UVA (40 J/cm²) irradiated 100 μl solutions evoked erythematous and indurated forearm responses at 24 h. Histology demonstrated epidermal polymorph accumulation with substratum corneum microabscesses. UVB (1500 mJ/cm²) irradiated LTB_4 failed to demonstrate these clinical and histological features. The mechanisms involved in UVB phototherapy of psoriasis are not known. Our results suggest that UVB-induced changes of the epidermal chemoattractant LTB_4 may play a role in this therapy.

Reduction of anthralin inflammation by application of topical amine compounds. B.Ramsay, C.M.Lawrence, J.M.Bruce* and S.Shuster, *Department of Dermatology, University of Newcastle upon Tyne and *Department of Chemistry, University of Manchester, U.K.*

Short contact anthralin therapy for psoriasis is often limited by inflammation. We have previously shown that anthralin inflammation requires the presence of anthralin in the stratum corneum for 24 h whereas the therapeutic effect requires contact for only minutes; anthralin inflammation can therefore be inhibited by inactivation with 1% KOH without affecting its therapeutic effect. Since KOH can itself be irritating, a series of less irritant lipid-soluble amines were studied to see if they could also inhibit anthralin inflammation. A total of 23 different primary, secondary and tertiary amines were assessed in 3 studies using 8 normal subjects in each study. Anthralin inflammation was produced by pipetting 20 μg anthralin in chloroform onto the skin. This was followed by the amine, equimolar with 1% KOH and dissolved in dichloromethane, dichloromethane alone, and 1% KOH; one anthralin site was left untreated. Inflammation was measured 48 h later by the increase in skin thickness, using Harpenden calipers. Anthralin inflammation was inhibited by 1% KOH ($P < 0{\cdot}05$) but not by dichloromethane alone. The following significantly reduced the inflammatory response at 48 h compared with the dichloromethane control site; the primary amines, ethanolamine, DL-1-amino-2-propanol, octadecylamine, octylamine; the secondary amines, diisopropanolamine, dihexylamine; and the tertiary amines, trioctylamine, triethanolamine and trihexylamine ($P < 0{\cdot}05$). Inhibition of anthralin inflammation by the amines was of the same degree as KOH and presumably by a similar mechanism of chemical inactivation. Thus, it may be possible to use these or similar amines to reduced anthralin inflammation without affecting its therapeutic effect.

A serological mediator in chronic urticaria. C.E.H.Grattan, *Department of Dermatology, The General Hospital, Birmingham, U.K.*

A previous study (Grattan *et al.*, *Br J Dermatol*, 1986; 114: 583) showed evidence of a circulating inflammatory mediator in the serum of some patients with chronic urticaria. The present study was undertaken to define better the nature of the mediator.

Fourteen patients with chronic urticaria and three control subjects were skin tested with autologous whole serum. Ten patients were also skin tested with whole autologous plasma and four patients were tested with autologous heat-treated (complement deficient) serum. Seven patients reacted with a wheal to their whole serum within 2 h of intradermal injection but none of the controls responded. Of the four serum-positive patients who were also tested with autologous plasma, all gave a positive response but none of the serum-negative patients reacted. Of the three serum-positive patients tested with autologous heat-treated serum, all reacted strongly but one serum-negative patient and one control subject were negative.

The sera of six patients and three controls were filtered through ultrafiltration membranes of 1,000 and 30,000 dalton nominal pore size. None of four whole serum-positive responders reacted to the 1,000 m.w. filtrate, three reacted to the 30,000 m.w. filtrate and all reacted to the residual autologous concentrates. Of the two patients who showed no response to whole serum, one reacted to both concentrates but neither reacted to the filtrates. None of the fractions from control subjects gave a positive response.

It is concluded that a serological mediator can be detected in some patients with chronic urticaria by its biological activity on skin testing. The evidence presented suggests that the mediator has at least one biologically-active component with a molecular weight between 1,000 and 30,000 daltons.

Interleukin 1 and neutrophil chemokinetic activity produced by human monocyte preparations. N.J.Fincham, R.D.R.Camp, F.M.Cunningham, A.J.H.Gearing* and C.R.Bird*, *Institute of Dermatology, St. Thomas's Hospital, London and *National Institute for Biological Standards and Control, Blanche Lane, South Mimms, Herts, U.K.*

Epidermal cell and monocyte derived interleukin 1 (IL-1) preparations have been reported to possess neutrophil chemoattractant properties *in vitro* (Luger *et al.*, *J. Immunol.*, 1983; **131:** 816). In view of a report that recombinant IL-1 was inactive in an agarose microdroplet neutrophil chemokinesis assay (Watson *et al.*, *Br J Pharmacol*, in press), we have re-investigated the nature of the chemoattractant material produced by lipopolysaccharide (LPS) stimulated monocyte preparations.

Human buffy coat monocytes were purified by density gradient centrifugation and plastic adherence, and the adherent cells (approximately 80% monocytes) cultured for 48 h as monolayers in RPMI 1640 containing 1 μg/ml *E. coli* LPS and 5% foetal calf serum. Medium (450 ml) was successively ultrafiltered to obtain a 10–30 kd fraction which was purified by reversed phase HPLC. Analysis of a portion (10 μl) of each 1 min fraction by the agarose microdroplet neutrophil chemokinesis method and by an IL-1 bioassay incorporating EL-4 NOB-1 and CTLL cell lines (Gearing *et al.*, *J Immunol Methods* 1987; **99:** 7) showed a discrete peak of chemokinetic activity eluting at 12–13 min, which was partly resolved from a broader peak of IL-1 activity (13–16 min). Further purification of the chemokinetic material by anion exchange HPLC, using a Tris acetate pH gradient, showed a peak of chemokinetic activity at 20 min (approximate pI = 5), containing a trace of IL-1 activity. Repurification of this material by reversed phase HPLC and assay of 0·5 min fractions now showed a peak of activity which was free of detectable IL-1 but produced dilution related chemokinetic responses.

These results indicate that human monocyte preparations produce a novel chemokinetic compound which may contaminate monocyte derived IL-1 which is not rigorously purified. This may explain in part the neutrophil chemoattractant activity ascribed to such IL-1 preparations.

Release of mitogenic cytokinesis by normal and psoriatic leukocytes. G.C.Priestley, *Department of Dermatology, University of Edinburgh, Edinburgh, U.K.*

Mitogenic effects of cytokines released by leukocytes could explain some characteristics of psoriasis (Kreuger & Jederberg, *J Invest Dermatol* 1980; **74:** 148). To study this possibility, venous blood from 12 healthy adults and from 12 patients with active plaque psoriasis was divided into mononuclear and polymorphonuclear (MN and PMN) cell fractions by centrifugation on Poly/mono resolving medium. The cells were incubated for 3 days in RPMI 1640 containing 10% foetal calf serum but no additional mitogens. Cell-free supernatants (S) were then pooled, diluted and fed to monolayers of logarithmically-growing skin fibroblasts.

In 6 lines of fibroblasts receiving undiluted MNS from the controls, proliferation was stimulated by 343 ± 125% (mean ± SEM) with a concentration-related effect ($r = 0·426$; $P < 0·01$) down to 68 ± 23% at 1/32 dilution. Undiluted MNS from the patients were less mitogenic (94 ± 61%) and the effect depended less on concentration ($r = 0·157$; $P > 0·1$). PMNS were less effective than MNS but those from controls were slightly more potent than those from patients (maximum 77 ± 13% against 54 ± 20%). Proliferation of NCTC 2544 skin epithelial cells was stimulated by up to 90% by control MNS but by less than 54% by those from the patients. The rank order of the mitogenic effect of fibroblasts, (control MNS > psoriatic MNS > control PMNS > psoriatic PMNS), was not explained by the interleukin-1 contents of the supernatants, which were greatest in those from the patients' cells (0·2 ng/ml). Interleukin-1 added to fibroblast cultures at 0·1–100 ng/ml stimulated proliferation by up to 40%. Dialysis of the mononuclear supernatants further increased the effect of those from the controls but decreased the stimulation by those from the patients.

These data confirm that leukocyte cytokines, especially those from mononuclear leukocytes, can exert a powerful influence on the skin but do not support the idea that they play a simple role in the pathogenesis of psoriasis.

The influence of gamma-interferon on the phagocytosis and killing of fungi by human macrophages and polymorphonuclear cells, D.C.KALTER*, R.A.CALDERON AND R.J.HAY*, **St John's Hospital for Diseases of the Skin, and Department of Medical Microbiology, London School of Hygiene and Tropical Medicine, London, U.K.*

Experimental evidence suggests a role for gamma-interferon (γ-IFN) in the enhancement of fungal phagocytosis and killing by mouse and human macrophages (Brummer *et al.*, *Inf Immun* 1985; **49:** 724). To examine its role in the handling of *Candida*, peripheral blood was obtained from healthy human volunteers and polymorphonuclear cells (PMNs) and monocytes (M) were collected via Ficoll and Percoll gradients. Each cell type, resuspended in modified RPMI at approximately 5×10^6 cells/ml, was incubated for $\frac{1}{2}$ to 24 h at 37°C with 5% CO_2 with α-IFN or γ-IFN, at concentrations ranging from 0·1 to 10^4 units/ml. *C. albicans* (C), reference strain NRB in yeast phase, was added to each well in a concentration of 10^4 cells/ml and incubated for 2 h at 37°C with 5% CO_2. Aliqouts from each resuspended well contents were cultured on NGP agar for 24–48 h at 34°C to assess the number of *Candida* colony forming units (CFU). Each experiment was repeated at least twice, with 4–8 replicates of each combination. Average CFUs were as follows:

C control = 117; C + α-IFN = 110; C + γ-IFN = 112; C + PMNs = 32; C + PMNs + α-IFN = 24; C + PMNs + γ-IFN = 10; C + M = 39; C + M + α-IFN = 38; C + M + γ-IFN = 8.

The results indicate a 2–3 fold increase in fungal killing by cells exposed to γ-IFN. PMNs were superior killers to Ms, regardless of the presence of γ-IFN; however, γ-IFN also enhanced PMN activity. This action on PMNs has only recently been suggested (Djeu *et al.*, *J Immunol* 1986; **137:** 2980). Optimal stimulation occurred at doses of 10–100 units/ml and after 24 h incubation of cells with γ-IFN. Current findings support a role for γ-IFN in the augmentation of host defence mechanisms against *Candida.*

Prevention of exudative epidermitis in Gnotobiotic piglets by bacterial antagonism. D.H.LLOYD, R.P.ALLAKER, I.M.SMITH AND A.MACKIE, *Department of Microbiology and Parasitology, Royal Veterinary College, London. U.K.*

We have chosen *Staphylococcus hyicus*, which causes porcine exudative epidermitis, as a model pathogen for studies of bacterial antagonism.

Six pairs of two-week-old piglets, in isolators, were inoculated in the sacral region with swabs saturated in overnight broth cultures of isolates P411, P119 or P170/SK. They were observed daily and swab samples were obtained from the skin caudal to the ears, the axillae and the sacrum at 1, 2, 5, 7, 13 and 16 days afterwards. The swabs were shaken in buffered Triton × 100 solution and the released bacteria estimated by culture on blood agar base by the spread plate method. In a subsequent study 4 piglets were used. P170/SK was inoculated onto one pair at 12 days of age and both pairs were inoculated with P411 two days later. Swab samples were obtained as before but the axilla was not sampled.

Piglets inoculated with P411 or P119 alone developed exudative epidermitis which first appeared 4 days after inoculation and became severe and extensive after 8 days. The development of lesions paralleled the skin bacterial populations. Lesions appeared when the number of cfu/swab exceeded 10^6; deaths occurred when counts reached 10^8. Piglets inoculated with P170/SK alone did not develop lesions and swab counts remained below 5×10^4. Those inoculated with P170/SK and P411 also failed to develop signs of exudative epidermitis. P170/Sk counts were similar to those obtained previously but counts of P411 remained below 10^4 per swab.

These studies show that avirulent *S. hyicus* is capable of antagonizing a virulent strain and preventing exudative epidermitis. The model provides a means by which staphylococcal disease and bacterial interference mechanisms can be conveniently studied.

The importance of staphylococcal colonization in atopic dermatitis. F.MORTON, R.S.LEVER, K.M.HADLEY* AND R.M.MACKIE, *Department of Dermatology, University of Glasgow and *Department of Bacteriology, Western Infirmary, Glasgow, U.K.*

Staphylococcal infection of the skin is a common complication in atopic dermatitis (AD). It is well recognized that there is high skin colonization with *Staphylococcus aureus* in this condition and it is isolated as the predominant organism in both involved and uninvolved clinically normal skin of these patients. The reason for this increased carriage is unclear although staphylococcal infections do appear to be associated with exacerbations of the disease. Forty-nine patients were entered into a double blind cross-over study of the use of mupirocin, a new topical antibiotic, in the treatment of AD. Four were withdrawn from the study and full bacteriological data are available for 42 patients. *S. aureus* was isolated from 36 of 42 (86%) after the run-in period (week 0). After two weeks topical treatment with mupirocin no *S. aureus* was found in 38 of 42 (90%). Clinical severity was monitored throughout the study and a significant improvement was seen after the mupirocin treatment. Phage-typing was carried out on all positive isolates. Two weeks after the mupirocin treatment was completed about half the patients had been recolonized. Less than half of these were recolonized with the same phage type.

This work was extended to study 38 children and 16 adults longitudinally over a longer period (up to 10 months). The carriage of *S. aureus* was 89% from areas of active dermatitis and 64% from clinically normal skin. In addition, a reduction in the number of *S. aureus* colonies was noted as the patients moved from a flare to a less active phase of the disease.

Seven patients with respiratory atopy but no cutaneous disease were also studied. No *S. aureus* was isolated. Thus, the carriage of *S. aureus* does not appear to be associated with the atopic state per se. The high frequency of *S. aureus* in AD could be related to the favourable microbiological environment provided by atopic skin, or, possibly, to abnormal adherence to the corneocytes. Further studies are in progress.

The effect of isotretinoin on the pilosebaceous duct in patients with acne. BRONWYN R.HUGHES AND W.J.CUNLIFFE, *Leeds Foundation of Dermatological Research, Leeds General Infirmary, Leeds, U.K.*

Isotretinoin is a proven treatment for acne and its main mechanism of action is thought to be a reduction in sebum production. It is also known to reduce comedones; such an effect may be a direct one on epidermal ductal differentiation or as a secondary event through changes in sebum composition.

We have attempted to answer this uncertainty by temporally relating changes in sebum excretion with changes in duct cornification.

Ten patients, (7 male and three female) mean age 20 years with moderate acne were treated with isotretinoin (1·0 mg/kg per day). Measurements were taken at weekly intervals of sebum excretion rate, and of microcomedones from the face and back using skin surface biopsies. The sebum excretion rate fell at a constant rate from 1·5 to 0·36 $\mu g/cm^2/min$. Analysis of the group as a whole showed that the number of microcomedones remained constant during the first month then decreased from the fourth to the eighth week by 1 log unit. However, detailed analysis of the data revealed 2 subpopulations in terms of microcomedone response. Six of the 10 subjects showed, during the first 2 weeks of treatment, a 2-fold increase in the number of microcomedones, whilst the other 4 subjects showed a 40% reduction.

Just how many microcomedones our non-invasive sampling techniques removes depends upon the weakest point of adhesion within the duct. The data, however, suggests that isotretinoin in the first four weeks has a direct effect on comedogenesis by two mechanisms. One is an increased separation of comedones (comedolysis), the other being reduced comedone formation as indicated by a marked reduction of microcomedones in 4 of the 10 subjects investigated. In the second month of the study the effect of isotretinoin on comedogenesis could be secondary to the marked changes in sebum excretion. A reduction in sebum excretion rate would be associated probably with a reduction in squalene oxide and an increase in linoleic acid, both of which have recently been shown to be associated with comedogenesis. Whatever is the precise role of isotretinoin in influencing comedogenesis it would appear that it probably does affect comedogenesis independently of changes in sebum excretion rate.

Effect of 13-cis-retinoic acid and testosterone on isolated human sebaceous glands. J.RIDDEN AND T.KEALEY, *Nuffield Department of Clinical Biochemistry, Oxford University, John Radcliffe Hospital, Oxford, U.K.*

Human sebaceous glands were isolated by shearing (Kealey *et al.*, *Br J Dermatol* 1986; **114**: 181) and maintained for 7 days on cellulose acetate filters (0·45 μm) floating on supplemented Williams E Medium with or without testosterone (10 μg/ml) and 13-cis-retinoic acid, 10^{-6} M.

Rates of lipogenesis were determined from 2 mM [U-^{14}C] acetate (specific activity 0·83 μCi/nmol). Rates of protein synthesis and DNA synthesis were determined by exposure to [U-^{14}C] leucine (specific activity 2 μCi/mmol) and [methyl-^{3}H] thymidine (specific activity 91 μCi/nmol) respectively. The rate of lipogenesis from [U-^{14} C] acetate declined significantly from 571·5 ± 69·8 pmol/gl/h (mean ± SEM; $n = 10$) to 48·6 ± 11·9 pmols/gl/h ($n = 11$) following 7 days maintenance ($P < 0{\cdot}001$, Student's t-test). However, 10 μg/ml testosterone significantly reversed the fall to 178 ± 44·7 pmol/gl/h ($P < 0{\cdot}05$; $n = 10$). Conversely, 10^{-6} M 13-cis-retinoic acid significantly reversed this testosterone effect to 10·9 ± 1·3 pmol/gl/h ($n = 4$; $P < 0{\cdot}01$).

The rates of protein and DNA synthesis for freshly isolated sebaceous glands were 76·6 ± 8·3 pmol/mg/h ($n = 8$) and 2·94 ± 0·17 fmol/mg/h ($n = 8$), respectively. After 7 days maintenance these rates dropped significantly to 55 ± 5 pmol/mg/h for protein synthesis ($n = 8$; $P < 0{\cdot}05$) and 0·43 ± 0·07 pmol/mg/h for DNA ($n = 8$; $P < 0{\cdot}001$). Testosterone significantly reversed this to 80 ± 7 pmol/mg/h for protein synthesis ($n = 4$), but not for DNA synthesis 0·94 ± 0·17 fmol/mg/h ($n = 4$; $P < 0{\cdot}001$). Conversely, 13-cis-retinoic acid reversed the testosterone effect to 41·6 ± 3·0 pmol/mg/h for protein ($n = 4$; $P < 0{\cdot}01$) and 0·25 ± 0·07 pmol/mg/h for DNA ($n = 4$; $P < 0{\cdot}05$).

We have shown, therefore, that isolated human sebaceous glands provide a possible model for the effect of hormones and therapeutic agents in acne vulgaris.

The incorporation of ^{14}C-labelled precursors into lipid by isolated human sebaceous glands.

B.Middleton, I.Birdi and J.R.Marsden*, *Department of Biochemistry, University of Nottingham Medical School and *Department of Dermatology, Queen's Medical Centre, Nottingham, U.K.*

The major substrate for sebaceous lipogenesis is not known. To clarify this we have investigated the rate of synthesis of lipid using ^{14}C-labelled glucose, lactate and acetate in isolated human sebaceous glands.

Freshly isolated sebaceous glands synthesized lipid from 1-^{14}C-acetate at 361 ± 122 pmol/h/gland ($n = 3$). This rate was increased after overnight preincubation of the glands in MEM culture medium to 1218 ± 217 pmol/h/gland ($n = 3$) and the incorporation was linear with time for at least 9 h ($r = 0{\cdot}85$).

After overnight preincubation, isolated sebaceous glands from 5 individuals were incubated for 6 h with different lipid precursors as sole carbon source and the rate of lipogenesis determined. With U-^{14}C-glucose (4 mM) as sole substrate the glands synthesized total lipid at 429 ± 181 pmol/h/gland and when the ^{14}C-glucose was replaced by U-^{14}C-lactate the rate was 943 ± 551 pmol/h/gland. The rate of total lipid synthesis from 1-^{14}C-acetate was 1128 ± 435 pmol/h/gland. These rates of lipogenesis were independent of substrate concentration over the range 4–8 mM.

Comparison of rates of synthesis of individual lipids in the above experiments showed that squalene synthesis was 59 ± 18 pmol/h/gland with ^{14}C-glucose as sole precursor but that this was significantly increased to 373 ± 162 pmol/h/gland ($n = 3$) when ^{14}C-acetate was used instead ($P < 0{\cdot}02$). Rates of synthesis of triglyceride were unaffected: 307 ± 11 and 352 ± 189 pmol/h/gland from ^{14}C-glucose and ^{14}C-acetate, respectively.

We conclude that isolated human sebaceous glands can synthesize lipid at high rates from glucose, lactate or acetate but that the rate is greatest when acetate is sole carbon source. The composition of newly synthesized lipid is also dependent on the nature of the carbon source.

Cholesterol modulation of lipid fluidity in epidermal cells as a model for cell membrane maturation.

B.Bonnekoh, B.Thiele, S.Daefler*, G.R.F.Krüger and G.Mahrle. *Departments of Dermatology and *Pathology, University of Cologne, F.R.G.*

Recently it has been shown by electron spin resonance that membrane lipid fluidity (MLF) of bovine epidermal cells (EC) decreases as the cells approach the horny layer (Tanaka *et al.*, *J Invest Dermatol* 1986; **87**: 745). This is paralleled by an increasing cholesterol content in epidermal cell membranes undergoing maturation (Gray *et al.*, *J Invest Dermatol* 1978; **70**: 233). The present study demonstrates that it is possible to modify the MLF of human (HEC) and guinea-pig epidermal cells (GPEC) *in vitro* by variation of external cholesterol.

Cholesteryl hemisuccinate in ethanolic stock solution (15 mg/ml) was diluted 1:100 into a solution of 3·5% polyvinylpyrrolidone, 1·0% bovine serum albumin and 0·5% glucose in phosphate-buffered saline. The resultant dispersion was centrifuged (1800 *g* for 20 min) in order to remove crystalline particles. EC were incubated either in this dispersion, in an ethanolic control mixture without cholesterol, or in pure McCoy's 5A medium at 37°C for 2·5 h. After incorporation of lipophilic diphenyl-hexatriene into the cells the degree of fluorescence polarization (*P* value, reciprocal of MLF) was determined at a concentration of 550,000 cells/ml at 25°C ($n=6$):

	Preincubation	Medium	Ethanol	Cholesterol
GPEC	0·277 ± 0·008	0·272 ± 0·009	0·283 ± 0·006	0·315 ± 0·008
HEC	0·278 ± 0·004	0·270 ± 0·002	0·280 ± 0·009	0·320 ± 0·003

MLF was significantly decreased with cholesterol in comparison with the three control conditions. On the other hand, steroids such as hydrocortisone-acetate and -hemisuccinate had no effect on the MLF of epidermal cells. Thus, it might be possible to influence EC maturation, at least at the cell membrane level, by variation of the lipid supply.

Survival of sex-mismatched cultured allogenic keratinocytes in the treatment of shaved tattoos assessed by a biotinylated Y probe. ANNE BRAIN, IRENE LEIGH, P.COATES AND TRISHA PURKIS, *Experimental Dermatology Laboratory, London Hospital, London, U.K.*

Previous reports (Thivolet *et al., Arch Dermatol Res* 1986; **278**: 252) have suggested that cultured allogenic keratinocytes are not rejected when used to graft epidermal wounds. We have previously shown accelerated wound healing by the use of cultured allogenic keratinocytes to graft leg ulcers and plastic surgery patients (Brain *et al., J Plastic Reconstructive Surgery*, in press). We have used in situ hybridization with a biotinylated Y probe to assess survival time of sex-mismatched cultured allogenic keratinocytes when used as grafts after removal of tattoos by shaving. Fifteen female tattoos were grafted with male skin and 9 male tattoos with female skin. Post-graft biopsies were taken at 1 week, 2 weeks, and 3 weeks post grafting. There was no evidence of survival of male cells in the biopsies of the female recipients and the percentage of male cells in the biopsies of male recipients was within the normal range. Therefore, there is no evidence of survival of allogenic keratinocytes from 1 week after their use as skin grafts and beneficial results must result from a biological dressing effect.

Abnormal retention of desmosomes in psoriatic scale. CHRISTINE J. SKERROW, DANAIDH CLELLAND AND D.SKERROW, *Department of Dermatology, University of Glasgow, U.K.*

It has long been suggested that abnormalities in the adhesive properties of keratinocytes contribute to the defective desquamation found in diseases such as psoriasis. This has been attributed to various alterations to the corneocyte surface, such as an increase in microvilli and changes in the lipid or carbohydrate composition; however, a direct role for these entities in keratinocyte adhesion has not been demonstrated. Desmosomes are intercellular junctions known to be of major importance in keratinocyte adhesion, but as yet no studies on the possible role of desmosomal abnormalities in scale formation have been carried out.

We have therefore investigated alterations to desmosomal components late in differentiation in normal and psoriatic tissue, using antibodies to both cytoplasmic and membrane-associated desmosomal proteins, and lectins which interact with glycosylated portions of the latter. Bound antibodies and lectins were visualized by immunogold electron microscopy. Results showed that, in normal epidermis, desmosomes persisted only into the inner stratum corneum, with reactivity to anti-desmosomal protein antibodies being lost immediately prior to the disintegration of their residual structures. This loss coincided with the point at which inter-corneocyte adhesion became susceptible to lipid solvents. In contrast, desmosomal components persisted into the uppermost layers of psoriatic scale, with reactivity for both antibodies and lectins being visible 40–50 cell layers removed from the living epidermis. In both cases, components of desmosomes linking the interdigitating ends of the horny cells were retained to higher levels than were those on the horizontal surfaces.

These results demonstrate that in normal inner stratum corneum, a transition occurs from desmosomal to non-desmosomal adhesion, and that this transition is grossly delayed in psoriasis. The abnormal retention of desmosomes, a known adhesion organelle, could clearly account for the increased corneocyte adhesion found in this scaling disorder.

Amyloid P component is associated with colloid bodies in human skin and undergoes calcium-dependent binding to isolated keratin filament aggregates. H.Hintner, J.Booker, J.Ashworth, M.B.Pepys* and S.M.Breathnach, *Department of Medicine (Dermatology), Charing Cross and Westminster Medical School, London and *Immunological Medicine Unit, Royal Postgraduate Medical School, London, U.K.*

Dermal colloid/keratin bodies, which are composed mainly of keratin intermediate filament (KIF) aggregates, are found in normal human skin due to apoptosis, and occur in increased quantities as a result of keratinocyte degeneration in certain lymphocyte-mediated skin disorders and primary localized cutaneous amyloidosis (PLCA). They are regularly coated with IgM anti-KIF autoantibodies. Colloid bodies are normally rapidly removed, but in PLCA they are converted by an unknown mechanism to amyloid. Amyloid P component (AP), a glycoprotein identical to the normal plasma protein serum amyloid P component (SAP), which shows specific Ca^{++}-dependent binding to certain ligands, is present in all forms of amyloid including PLCA. We have therefore investigated the interaction between AP/SAP, colloid bodies and KIF aggregates.

Cryostat sections of biopsies of normal skin from 6 subjects were stained by a double immunofluorescence technique using fluoresceinated anti-SAP, and either rhodamine-conjugated anti-IgM, or AE-1/AE-3 anti-keratin antibodies followed by species-specific texas red-conjugated anti-mouse immunoglobulin, to identify colloid bodies. The percentage (mean ± SEM) of colloid bodies which bound anti-SAP was 52 ± 4%. Isolated KIF aggregates, extracted from normal human epidermis using 8M urea, were incubated with normal human serum as a source of SAP in the presence of Ca^{++}; control incubations were performed in the presence of EDTA. The aggregates showed bright fluorescence with anti-SAP only when the incubation medium contained Ca^{++}. The specificity of the calcium-dependent SAP binding to KIF aggregates was confirmed in studies in which aggregates preincubated with serum were analysed using SDS-PAGE, followed by immunoblotting to identify SAP. Since it has been suggested that persistence of colloid bodies may favour their conversion to amyloid in PLCA, it was of interest to determine whether AP bound to KIF aggregates prevented their digestion by proteinases. KIF aggregates were pre-incubated with a large excess of human serum, in the presence of either Ca^{++} or EDTA, and exposed to trypsin or alpha-chymotrypsin at optimal concentrations for defined periods; the reactions were monitored by SDS-PAGE. Calcium-dependent binding of SAP to KIF aggregates, confirmed by immunofluorescence and immunoblotting, did not alter the degree of keratin filament degradation. Our results demonstrate that AP is associated with colloid bodies *in vivo* and binds in a calcium-dependent fashion to KIF aggregates *in vitro*. These findings indicate that keratin filaments represent a newly recognized ligand for AP/SAP.

Novel terminal glycosylation of blood group substances during embryogenesis of the skin. S.Fleming and Susan E.Kelly*, *University Department of Pathology, Southampton General Hospital, Southampton and *Department of Dermatology, Slade Hospital, Oxford, U.K.*

Different fucosyl poly-N acetyl lactosamine derivatives confer blood group and related antigenic specificities. Glycoconjugates of this type have been shown to be important at different stages of embryogenesis. We have investigated the expression of these molecules during the embryogenesis of the skin in the rat by using monoclonal antibodies. The primitive ectoderm expressed only the Le^x

determinant, but with further maturation this reactivity was lost. Blood group substance H (Type II) and Le^y were not present. Specific glycosyl hydrolases were used to analyse the chain structure of the antigens in the fetal skin. Sensitivity of Le^x expression to glycopeptidase F suggests this is carried on an N-linked oligosaccharide. Surprisingly, cryptic blood group H specificity in skin from 15 days onwards was unmasked by treatment with endogalactosidase. This shows that H substance is rendered cryptic by a linear non-fucosylated extension of the poly-N acetyl lactosamine core. This novel form of modifying these molecules is important in the regulation of their function *in vivo* and in their detection by immunological methods.

6-Thioguanine-resistant mutant frequency is elevated in the circulating T lymphocytes of xeroderma pigmentosum. J.Cole, C.F.Arlett and M.H.L. Green, *M.R.C. Cell Mutation Unit, University of Sussex, Falmer, Brighton, Sussex, U.K.*

We have recently developed techniques whereby circulating T lymphocytes may be established *in vitro* with high cloning efficiencies, permitting precise estimates of the frequency of 6-thioguanine-resistant mutants. Amongst normal individuals there is an age-related increase in mutant frequency $\sim 5 \times 10^{-7}$ in cord blood to in excess of 10^{-5} at age 80 years, the major increase occurring during the first two decades of life. Mutant frequencies from patients suffering from the sun-sensitive, cancer-prone autosomal recessive disease xeroderma pigmentosum (XP) and some heterozygotes has been determined.

Patient	Complementation group	Age (years)	Mutant frequency per colony-forming cell
1	D?	7	4·09
2	G	15	7·74
3 (sib of 2)	G	12	3·63
4a	D	32	24·24
4b	second sample	36	32·59
5	Variant	51	40·30
6	D	13	40·57

When tested against the appropriate controls from the data base, and taking smoking and age into account, the mutant frequency in XP is significantly higher than controls by a factor of 4. These results with T lymphocytes confirm the enhanced mutability of fibroblast cultures established from XP patients when exposed to 254 nm UV radiation. It is not known whether the increased mutant frequency seen in T cells is a reflection of an intrinsic spontaneous mutability caused by defects in DNA repair or the consequence of exposure of the cells to sunlight during transit in the skin.

DNA damage associated with azathioprine and UVR in renal transplant recipients. Ann Dalton*, C.I.Harrington†, D.Curtis*, J.L.Maddocks†, L.Lennard†. **Centre for Human Genetics, Sheffield, and* †*Royal Hallamshire Hospital, Sheffield, U.K.*

We have previously shown that renal transplant recipients (RTRs) who develop premalignant and malignant skin lesions have raised levels of the azathioprine metabolite 6-thioguanine nucleotide (6-TGN) compared with RTRs who have not developed cutaneous abnormalities (Lennard *et al.*, *Br J Dermatol* 1985; 113: 723). It has been suggested that patients on azathioprine have raised levels of sister chromatid exchange (SCE). Therefore, this study was undertaken to determine whether incorporation of 6-TGN into DNA, together with UVR, lead to DNA damage that might be responsible for the development of cutaneous malignancy as measured by rates of SCE.

Patients were matched for age, sex, transplant duration, azathioprine dosage, skin type and UVR exposure. Groups studied were (i) RTRs with lesions, (ii) RTRs without cutaneous lesions (iii) patients receiving azathioprine for chronic dermatoses, (iv) normal controls.

Preliminary results showed that all patients on azathioprine had an increased baseline level of SCE compared with controls ($P=0{\cdot}005$). All groups showed a significantly increased level of SCE in response to UVR (controls $P=0{\cdot}005$; skin conditions $P=0{\cdot}025$; RTRs $P=0{\cdot}005$) but the RTR group showed a significantly greater response than the two other groups ($P=0{\cdot}005$).

Cellular sensitivity to UVA in photosensitivity dermatitis/actinic reticuloid (PD/AR).

B.E. Johnson, E.M.Walker, J.Ferguson and W.Frain-Bell, *Department of Dermatology, University of Dundee, Dundee, U.K.*

Cells from subjects with actinic reticuloid are reported to be more UVA sensitive than those from normals (Botcherby *et al., Photochem Photobiol* 1984; **39**: 641). We have therefore compared the UVA sensitivity of fibroblasts from 18 subjects with clinical, phototest and patch test evidence for the diagnosis of photosensitivity dermatitis/actinic reticuloid (PD/AR) with that of cells from 16 non-photosensitive controls. Established cell lines were grown to confluence in 40 × 65 mm Falcon flasks and fields 8 mm in diameter were exposed *in situ* at room temperature to a series of doses at 335 ± 30 nm and 365 ± 30 nm from an irradiation monochromator. After 24 h at 37°C the cultures were washed, fixed in formol-saline and stained with Giemsa. The exposure dose for cell killing (MLD) was determined as that which resulted in a stain-free zone in the culture. Identical results were obtained with the eosin exclusion test. There was a wide range of UVA sensitivity in PD/AR cells but an equal range for cells from controls (Table).

Table

		MLD (J/cm^2)						MED (J/cm^2) (range)	
Subjects	No.	335 nm: Mean	SEM	range	365 nm: Mean	SEM	range	335 nm	365 nm
PD/AR	18	8·9	1·5	2·5–24	17·7	2·8	4–48	0·05–2·2	0·1–11
Controls	16	11·7	1·4	1–32	23·1	2·6	3–48		

There is no significant difference in MLD for the two groups at either waveband ($P > 0{\cdot}2$). There was no correlation between the severity of skin disease at the time of biopsy and the UVA sensitivity of the PD/AR cells. Similarly, there is no correlation between the minimal erythema dose (MED) at 335 ± 30 nm or 365 ± 30 nm determined at that time and the MLDs for the fibroblasts. Correlation coefficients: MED/MLD335 = −0·253; MED/MLD365 = −0·271. There appears to be a wide general variation in fibroblast sensitivity to UVA which encompasses that of actinic reticuloid cells. However, it is possible that PD/AR does consist of subsets of subjects with varying inherent cellular sensitivity which has no relationship to skin reactions or disease process. Moreover, some non-photosensitive subjects provide fibroblasts with extreme sensitivity to UVA of as yet unknown aetiology.

The effect of topical indomethacin on ultraviolet erythema in polymorphic light eruption.

P.M.Farr and B.L.Diffey*, *Dermatology Department, Royal Victoria Infirmary, Newcastle upon Tyne and *Regional Medical Physics Department, Dryburn Hospital, Durham, U.K.*

Cyclo-oxygenase inhibition by topical indomethacin reduces the intensity of ultraviolet (UV) erythema in normal skin but augments the response in actinic prurigo (Farr & Diffey, *Br J Dermatol*, 1987; **116**: 425). Since polymorphic light eruption (PLE) and actinic prurigo have several similar features, and may be difficult to distinguish clinically, we studied the effect of indomethacin on UV erythema in PLE.

Ten females (aged 24–44 years) with PLE were studied and all had normal 24 h UVB and UVA minimal erythema responses. Sites 1 cm diameter on the uninvolved skin of the back were exposed to UVC, UVB or UVA; 1% indomethacin gel (0·1 mg/cm²), or inactive gel base, was then applied to the skin and the increase in erythema measured with a reflectance instrument at 8 and 24 h.

UVC and *UVB*: In all patients, 8 and 24 h erythema was significantly reduced at the sites treated with indomethacin compared with the inactive gel base treated sites.

UV-A: 8 h erythema was reduced significantly by indomethacin but there was no significant difference between indomethacin and gel base treated sites at 24 h.

The inhibitory effect of indomethacin on UV erythema in PLE is identical to that found in normal subjects and differs from the augmentation found in actinic prurigo. These results show that PLE and actinic prurigo have a different mechanism of photosensitivity.

Polymorphic light eruption: an immunopathological study of evolving lesions. P.G.NORRIS, J.MORRIS*, A.C.CHU* AND J.L.M.HAWK, *Photobiology Unit, Institute of Dermatology, UMDS of Guy's and St. Thomas' Hospitals and *Department of Dermatology, Hammersmith Hospital, London, U.K.*

A cell-mediated immune mechanism (CMI) has been postulated in polymorphic light eruption (PLE) but direct evidence has been lacking, an immunopathological study of naturally-occurring PLE lesions of uncertain duration giving inconclusive results (Muhlbauer *et al.*, *Br J Dermatol*, 1983; **108:** 153). We have induced PLE in 9 patients 5–20 h after single exposures to 25–80% of their minimal erythema dose of solar simulated radiation; 4 mm punch biopsies from irradiated sites were snap frozen for immunohistochemistry. Dako-T4 (helper), Dako-T8 (suppressor), OKT6 (Langerhans cell (LC)), Dako-M (macrophage) and RFD1 (interdigitating reticulum cell) monoclonal antibodies were used in an APAAP technique. A T cell infiltrate, Dako-T4 cells outnumbering Dako-T8, initially perivascular and increasingly diffuse from 24 h, was first evident at 5 h and profuse by 72 h post-irradiation. A significant increase in dermal macrophages and LC was detected at 1 and 5 h, respectively (see Table). RFD1 +ve cells were not identified. Irradiated control subject skin showed no cell infiltration. Our findings suggest a CMI response may be casually associated with the pathogenesis of PLE.

TABLE

	Upper dermal cells/graticule area (mean ± SEM)				
	Pre-irradiation	1 h	5 h	24 h	72 h
Dako-T4	1·7±0·7	2·5±0·3	23·9±11·9	68·3±13·6*	76·6±16·5**
Dako-T8	0·8±0·6	0·5±0·5	15·7±8·4	49·4±12·3*	64·1±10·9**
OKT6	5·1±2·4	9·5±2·5	11·6±1·4*	10·8±2·9	6·6±2·2
Dako-M	8·6±0·4	19·1±0·1**	20·2±4·8	19·7±2·7**	19·5±4·0*

* $P<0{\cdot}01$, ** $P<0{\cdot}001$

A new method for expressing the action spectrum in drug-induced photosensitivity. B.L.DIFFEY AND P.M.FARR*, *Regional Medical Physics Department, Dryburn Hospital, Durham, and *Department of Dermatology, Royal Victoria Infirmary, Newcastle upon Tyne, U.K.*

In studies of photosensitivity induced by chemical agents, it is often presumed that if the agent itself is the photosensitizer, the absorption spectrum *in vitro* and action spectrum *in vivo* should be similar. In practice, the correspondence is often not as close as expected. In particular, studies have shown that many drugs appear to exert no photosensitizing potential at wavelengths below 320 nm (UVB and UVC) despite the fact that the drugs absorb these wavelengths strongly, and often the *in vivo* action spectrum extends to longer wavelengths beyond those which the drugs absorb.

We believe that the apparent discordance has arisen because of inappropriate treatment of the experimental data in which the underlying normal response of the skin to ultraviolet radiation has been ignored. If erythema is the end-point of the phototoxic response, we propose that the relative effectiveness of radiation of a given wavelength (the action spectrum σ) may be expressed as

$$\sigma = {}^{1}/[\text{Minimal phototoxic dose}] - {}^{1}/[\text{Minimal erythema dose in unsensitized skin}]$$

This expression was applied to our own experimental studies of photosensitivity induced by hydrochlorothiazide and quinine in human subjects. For each drug, the experimentally-observed *in vivo* action spectrum was entirely compatible with the *in vitro* absorption spectrum of the drug. This was not the case if the action spectrum is expressed by the reciprocal of the MPD, as is conventional practice.

We conclude that if the simple model proposed here is applied to the published data of many studies of drug-induced photosensitivity, much closer agreement between the absorption spectrum and corrected action spectrum would result.

UVB therapy prolongs remission of psoriasis after short contact dithranol therapy. Y.PARAMSOTHY, M.COLLINS* AND C.M.LAWRENCE, *Department of Dermatology and *Community Health Centre, North Staffordshire Hospital Centre, Stoke on Trent, U.K.*

We have investigated the effect of adding UVB therapy to short contact dithranol therapy (SCDT). Fifty-three patients with psoriasis (21 females and 32 males, mean age 44·8 years, range 16–88 years) were stratified and randomly allocated so that 27 patients received a tar bath and UVB therapy and 26 patients received an emulsifying ointment bath only. All patients received 20 min applications of dithranol 1–16%. UVB therapy was given daily so that redness was achieved without burning, using a Waldmann 1000 Unit with 26 Waldmann F85/100W/UV6 fluorescent lamps, which emits UVB+UVA in a wavelength range of 285–350 nm, maximal at 310–315 nm.

Five patients in the non-UVB group withdrew. In the UVB-treated group, 20 of the 27 patients cleared in 20·3 ± 1·6 days (mean ± 95·1% CI), compared with 16 of the 21 patients in the non-UVB treated group who cleared in 19·9 ± 2·6 days ($P > 0{\cdot}05$). There was no difference between the rate of decrease of the extent of the psoriasis, palpability, erythema, scaling or plaque thickness between the two groups ($P > 0{\cdot}05$). Thirteen of the 16 patients treated with dithranol alone relapsed in a mean time of 10·6 weeks and 3 remained clear at a mean of 42 weeks, compared with 14 of the 20 patients in the dithranol-UVB group who relapsed in a mean of 18·9 weeks and 6 remained clear at 30·8 weeks (unpaired *t*-test $P < 0{\cdot}05$). Full blood count, biochemical screen and urinalysis remained unchanged. Thus, UVB therapy does not improve the clearance of psoriasis in SCDT, but does significantly prevent relapse of psoriasis.

Effects of a helium neon laser on aspects of wound healing. G.B.COLVER, L.W.ADAMS, M.J.SPENCER AND G.C.PRIESTLEY, *Department of Dermatology, The Royal Infirmary, Edinburgh, U.K.*

Low power laser radiation may alter physiological processes. The red light of a helium neon (He Ne) laser appears to stimulate skin fibroblasts and epithelial cells and influence wound healing. We have studied human skin fibroblasts, epithelial and endothelial cell proliferation, collagen lattice contraction, cellular migration from normal skin explants and collagen synthesis. We used a 5 mW He Ne laser emitting a beam of maximum diameter 1 mm at a wavelength of 632·8 nm. The power of density was $\simeq 10\ \text{mW/mm}^2$ and the beam was 'brushed' over monolayer cultures to ensure equal exposure to all areas.

NCTC 2544 skin epithelial cells when irradiated 3 times daily for 5, 10, 30, or 60s yielded 8 ± 7%, 28 ± 17%, 26 ± 13% and 22 ± 11% greater proliferation than untreated controls (means from 4 cultures ± S.E.M., $P > 0{\cdot}1$). In a repeat experiment, the increases were within 10% of controls. The maximum increase in fibroblast proliferation was not significant ($P > 0{\cdot}5$). Endothelial cell proliferation was not affected in one experiment but was diminished in another. Explants of normal skin irradiated for 30 s (3 times) daily showed no clear effect on cellular migrations. Lattices of Type I collagen containing normal skin fibroblasts contracted at normal rates despite twice daily irradiation; no major morphological changes were seen in the irradiated cells by light or electron microscopy. Collagen synthesis was studied as the incorporation of ^{3}H-proline into collagenase-sensitive protein by confluent cultures of fibroblasts. The rate of synthesis was unaffected by He Ne irradiation (controls 24306 ± 944 dpm (mean ± SEM); irradiated 23931 ± 2419 dpm).

We have failed to identify significant effects of He Ne irradiation, but any clinical improvement in wound healing may derive from a pathway we have not examined. The effect of irradiation on fibroblast glycosaminoglycans production is currently being studied.

POSTERS

UVA dose response of normal and actinic reticuloid fibroblasts; a statistical analysis.

N.K.GIBBS, P.K.BOTCHERBY*, R.W.MORRIS†, A.R.YOUNG AND F.GIANNELLI*, *Institute of Dermatology, *Paediatric Research Unit and †Community Medicine, U.M.D.S., London, U.K.*

Actinic reticuloid (AR) subjects show photosensitivity which often extends into the UVA and visible spectra. *In vitro*, AR fibroblasts show UVA hypersensitivity (Botcherby *et al., Photochem Photobiol*, 1984; 39: 641). To quantify this sensitivity further a full statistical analysis of the UVA dose responses of 2 AR (AR4LO & AR6LO) and 3 normal fibroblast strains was conducted.

1×10^5 cells, in DMEM with 10% donor calf serum, were seeded into 30 mm dishes. At confluency, 5–6 days later, dishes were exposed to 40, 70 or 100 kJ/m² UVA from a 3 mm polystyrene filtered bank of 2 Sylvania F40 'black light blue' fluorescent tubes (irradiance $\sim 2 W/m^2$). Fresh medium was applied before and after irradiation; 24 h post-irradiation cell viability was assessed by uptake of 1 μCi/ml [^{3}H] hypoxanthine. In each of the 5 repeated experiments a UVA dose–response curve was obtained for all 5 strains. Great emphasis was placed on keeping conditions constant across experiments. Data was analysed in 2, 3 and 4 way ANOVAS using a GLIM package.

Across the 3 doses the 4-way ANOVA showed that the AR cells were more sensitive ($P < 0.001$); 3-way ANOVA suggested that this increased sensitivity was most apparent at the intermediate dose (70 kJ/m²) ($P < 0.01$). Within the AR and normal categories, there was no significant variation in strain response. When data from all dose–response curves was analysed in the 4-way ANOVA, inter-experiment differences were significant ($P < 0.01$).

This series of repeated experiments demonstates that 2 AR fibroblast strains were significantly more sensitive to UVA radiation than 3 normal strains. Under the conditions of our experiments, this increased sensitivity was most apparent in a certain 'dose window' around 70 kJ/m². (Supported by the National Radiological Protection Board, U.K.).

UVA and UVB induced changes of skin collagen in the hairless mouse.

J.A.HARRISON, S.R.PLASTOW AND A.R.YOUNG, *Photobiology Unit, Institute of Dermatology, Lambeth Hospital, London, U.K.*

Previous studies in the hairless mouse have shown an increase in the proportion of type III skin collagen after 12 weeks exposure to UVB (Plastow *et al., J Invest Dermatol* 1987; 88: 145). We have studied changes in type III collagen after exposure to fluorescent solar simulated radiation (FSSR which contains UVB and UVA) and UVA alone.

One group of 12 Skh-1 hairless albino mice was irradiated 8 h per weekday for 16 weeks with a daily dose of 20 J cm² UVA and 240 mJ cm² UVB (FSSR from 6 Phillips TL10 PUVA tubes and 2 Wolf Helaria UVB tubes). Another group was exposed to the same regimen but without the UVB tubes. A third group received 20 J cm² UVA per weekday for 32 weeks from the above UVA and UVB sources filtered by 12 mm window glass to eliminate any UVB. Each group was compared with its age and time matched non-irradiated control. At the end of the exposure regimens, skin samples were taken from dorsal and ventral sites. Types I and III collagen were quantified densitometrically after cyanogen bromide digestion and SDS PAGE. Type III was expressed as (III/III + I) × 100% and the ratio of dorsal/ventral type III (D/V III) collagen was determined for each mouse.

D/V III for the FSSR group was 1·28 which was significantly greater than the 0·99 control level ($P < 0.001$). D/V III for the 16-week UVA group was 1·14 which was not significantly different from the control ($P > 0.2$) but D/V III for the 32-week UVA group was 1·14 which was significantly greater than the

0·88 control level ($P < 0·001$). As previously observed, the control D/V III value decreased with age. These data provide quantitative biochemical evidence that UVA may be important in photoageing.

Mechanical strength of glutaraldehyde-crosslinked collagen suitable for use as a dermal implant. M.J.Raxworthy, C.M.Lachs and E.J.Wood, *Department of Biochemistry, University of Leeds, Leeds, U.K.*

There is currently much interest in the use of collagen as a biochemical for the replacement of dermal tissue following major cutaneous injury. Crosslinking with glutaraldehyde (GTA) reduces collagen biodegradation and antigenicity but tends to be leached from the implant causing local tissue damage. We have studied the abilities of sodium borohydride ($NaBH_4$) and L-lysine, both of which should influence the interactions between GTA and collagen, to modify the mechanical properties of crosslinked collagen.

Fibrous collagen from rat dorsal skin was prepared by treatment with crystalline trypsin for 28 days. After crosslinking by 0·01% GTA, collagen was incubated with 0·5% $NaBH_4$, 5 mM L-lysine or 0·5% $NaBH_4$ followed by 5 mM L-lysine. Collagen sheets of a standard thickness were cut to a dumbell shape of precise dimensions and stress-strain curves were obtained using an Instron testing machine. The low and high strain moduli (LSM and HSM, respectively) and the ultimate load (e_M) were determined ($n = 3$):

Table

Treatment	LSM	HSM	e_M
Non-crosslinked	1·3	2·9	0·92
GTA	2·2	7·1	0·38
GTA + $NaBH_4$	3·4	8·7	0·65
GTA + L-lysine	2·5	4·2	0·82
GTA + $NaBH_4$ + L-lysine	1·2	6·4	0·92

These parameters give a measure of the alignment of collagen fibrils in the matrix (LSM) and the straining of inter- and intramolecular crosslinks (HSM). While GTA crosslinking alone produced a stronger and more rigid (i.e. less elastic) material, subsequent treatment with $NaBH_4$ conferred greater strength and elasticity. Lysine-treated, crosslinked collagen was mechanically weaker but more elastic than crosslinked alone. After treatment with both $NaBH_4$ and lysine it had LSM and e_M values approaching those for non-crosslinked collagen whilst retaining a greater HSM.

Antibodies to collagen types I–V in granuloma annulare and necrobiosis lipoidica. C.D.Evans, R.S.Pereira*, C.T.Yuen and C.A.Holden, *Department of Dermatology and *Department of Immunology, St. Helier Hospital, Carshalton, Surrey, U.K.*

Granuloma annulare (GA) and necrobiosis lipoidica (NL) are characterized by focal degeneration of dermal collagen. Indirect evidence suggests that the primary event is a vasculitis due to circulating immunoreactants (Dahl, *Aust J Dermatol* 1985; **26:** 49). Bovine collagen implants sometimes produce a local reaction which resembles GA histologically (Barr *et al., J Am Acad Dermatol* 1982; **6:** 867) and serum IgG antibodies to the implant have been demonstrated in patients who react (McCoy *et al., Arch Dermatol* 1985; **12:** 990). We wished to determine whether auto-antibodies to collagen play a part in the pathogenesis of GA and NL.

We took serum samples from 32 patients with GA, of whom 4 were diabetic and from 32 patients with NL of whom 16 were diabetic. Sera from 31 healthy controls were selected randomly. Antibodies to native collagens I–V were measured by a standard enzyme linked immunosorbent assay method, in duplicate.

Anti-collagen antibodies were detected against collagen types I–IV in normal controls, GA and NL. We found very low levels of antibodies to type V collagen.

TABLE

Collagen type	Antibody levels: arbitrary units (mean ± SD)		
	GA patients	NL patients	Controls
I	0·52 ± 0·18	0·59 ± 0·15	0·58 ± 0·21
II	0·56 ± 0·20	0·57 ± 0·13	0·62 ± 0·16
III	0·37 ± 0·13	0·46 ± 0·16	0·51 ± 0·14
IV	0·36 ± 0·13	0·27 ± 0·10	0·35 ± 0·12
V	0·10 ± 0·05	0·03 ± 0·05	0·07 ± 0·05

The results demonstrate no increase in circulating IgG antibodies to collagens I–V in GA and NL. The data strongly suggest that circulating auto-antibodies to collagen play no part in the pathogenesis of GA or NL.

Studies of interactions between porcine skin bacteria. R.P.ALLAKER, D.H.LLOYD AND I.S.MASON, *Department of Microbiology and Parasitology, Royal Veterinary College, London, U.K.*

Interactions between *Staphylococcus hyicus*, the causative organism of exudative epidermitis of pigs, and antibiotic producing porcine skin bacteria (inhibitors) have been investigated to select strains which may reduce *S. hyicus* colonization *in vivo* and so prevent disease.

The inhibitors fall into 3 species groups; *S. epidermidis*, *S. hominis* and *Aerococcus viridans*. The effect on *S. hyicus* growth of a representative strain from each group was investigated. Antagonism was studied quantitatively using pure and mixed cultures grown on cellulose acetate membranes lying on blood agar base. Membranes were removed at intervals, washed and the recovered viable bacteria counted. The nature of their interactions were studied on Nucleopore membranes in the scanning electron microscope (SEM).

On acetate membranes all inhibitors reduced *S. hyicus* growth ($P < 0{\cdot}001$). *S. epidermidis* caused inhibition between 10 and 20 h post-inoculation; *S. hominis* started to inhibit at 10 h after which *S. hyicus* growth remained stationary. *A. viridans* induced immediate inhibition and *S. hyicus* was no longer isolated after 8 h. In the SEM, *S. hyicus* cells appeared collapsed in the presence of *S. epidermidis* and *S. hominis* but remained morphologically unchanged in the presence of *A. viridans*.

These studies demonstrate that inhibitors found on pig skin have different modes of action against *S. hyicus* and can greatly reduce its growth on membranes designed to simulate the skin surface. Related *in vivo* studies with a gnotobiotic pig-skin model have also shown inhibitory activity by these organisms (Lloyd & Allaker, unpublished). These models provide a means for further study of bacterial interaction in staphylococcal skin disease.

Vaccination and experimental *Dermatophilus congolensis* infection of rabbits: a model for factors influencing epidermal infection. S.J.HOW AND D.H.LLOYD, *Department of Medicine, Royal Veterinary College, North Mymms, Herts, U.K.*

Dermatophilus congolensis has been proposed as a model pathogen in mice for the investigation of factors influencing skin infection (Lloyd & Noble, *Br Vet J* 1982; **138:** 51). Here the use of *D. congolensis* infection in rabbits to examine effects of active immunity on epidermal challenge following vaccination is reported.

Rabbits were vaccinated intradermally with a live preparation of *D. congolensis* strain SS18C (group SSVAC) or FD11 (group FDVAC). A control group (UNVAC) was not vaccinated. Two weeks after vaccination, separate 1·5 cm^2 clipped and ether swabbed skin sites were inoculated with a 10-fold dilution range (10^7 to 10^1 zoospores/cm^2) of SS18C or FD11. Lesion scores at each site were calculated from the sum of individual scores for erythema, oedema and scab formation (0–4+) multiplied by the percentage of the area affected. Scores were compared for each group at each dose on each day by analysis of variance.

In groups SSVAC and FDVAC, lesions were less severe and developed more quickly than in the control group (UNVAC). For the vaccinated groups the number of zoospores/cm^2 of skin required to produce infection was 10^4-fold higher for sites inoculated with the vaccine homologous strain and 10^2-fold higher for the heterologous strain compared with the control sites. In the UNVAC group a clear dose–response relationship between the size of the inoculum and the severity of the lesions which developed was seen for both *D. congolensis* strains.

The dose–response relationship demonstrated using this model enabled semiquantitative analysis of the effects of vaccination on *D. congolensis* infective dose and severity of infection. It is proposed that this model could be used to carry out semiquantitative studies on the effects of other factors, such as chemotherapy, on skin infection.

Patients with eczema herpeticum have prolonged depression of cell-mediated immunity to herpes simplex virus. J.P.Vestey*, S.E.M.Howie, M.Norval and J.P.Maingay. *Departments of *Dermatology and Bacteriology, University of Edinburgh, Edinburgh, U.K.*

A survey of 58 patients with primary and recrudescent facial herpes simplex virus (HSV) lesions was undertaken to study the immune response to the virus. Nine patients with atopic eczema and 7 with eczema herpeticum (EH) were included. Antibody levels to HSV type 1 were measured by an enzyme-linked immunosorbent assay, and cell-mediated immunity (CMI) to HSV type 1 by *in vitro* lymphocyte proliferation. All patients had consistently high antibody titres and all but 3 had positive CMI. In 23 cases the CMI response decreased with time from the last recrudescent lesion but always remained positive. Thirteen control subjects were negative for antibody and CMI to HSV.

The 3 patients with negative CMI had suffered extensive eczema herpeticum affecting greater than 25% body surface area. The response remained negative for periods of 5, 6 and 11 months, respectively, before gradual recovery of a normal response. In the third case, 7 months after EH, the T8-positive cells were removed by panning before setting up the lymphoproliferation assay and this procedure restored the CMI response to HSV to a normal level.

It is concluded that patients with severe EH may have profoundly depressed CMI to HSV for several months, possibly because of an over-active T suppressor cell response.

A rapid immunofluorescent technique for identifying herpes simplex virus and varicella zoster virus in vesicular skin lesions. P.Grint*, I.H.Coulson† and P.Watkins‡, **Department of Virology, St Bartholomews Hospital, London and Departments of †Dermatology and ‡Virology, St. George's Hospital, London, U.K.*

In the immunocompetent, 15% of vesicular zosteriform lesions are due to herpes simplex virus (HSV) rather than varicella zoster virus (VZV) (Kalman & Laskin, *Am Med J* 1986; **81:** 775). In the immunocompromised, lesions may be more atypical, and prompt therapy may be desirable. As choice of antiviral therapy or its dose may differ for different herpes virus infections a technique that rapidly distinguishes HSV from VZV is required.

Over 100 specimens have been examined from patients with chickenpox, shingles, eczema herpeticum and other herpes simplex skin infections. Vesicle scrapings were examined by indirect immunofluorescence (IF) using novel mouse monoclonal antibodies to HSV internal type common proteins and VZV internal protein which give distinctive nuclear staining in herpes virus infected cells. Electron microscopy (EM) of vesicle fluid and tissue culture of lesion swabs were performed in parallel.

Over 90% of herpetic lesions gave a specific positive result for either HSV or VZV by IF. IF and EM gave a similar number of positive results. A greater number of specimens was positive by IF than by culture. All 20 negative control specimens taken from patients with non viral vesico-bullous lesions (pemphigoid, pompholyx and cryotherapy blisters) were negative by IF, EM and culture.

Using these two antibodies, IF is a rapid, reliable, inexpensive and, more importantly, virus-specific technique for identifying HSV and VZV.

Acne vulgaris and malignancy. R.A.SHEEHAN-DARE, W.J.CUNLIFFE, A.V.SIMMONS‡, J.A.CHILD*, P.A.MCKINNEY†, AND R.A.CARTWRIGHT† *Department of Dermatology, *Department of Haematology, and *Leukaemia Research Fund Centre for Clinical Epidemiology, Department of Pathology, The General Infirmary, Leeds and ‡Department of Medicine, St. James University Hospital, Leeds, U.K.*

Propionibacterium acnes (*P. acnes*) has immune-stimulatory and anticancer properties in animals (Scott, *Semin Oncol*; 1974; **1:** 367). It is possible that *P. acnes* has similar effects in humans, particularly subjects with acne where high numbers of *P. acnes* may be present (Marples *et al.*, *J Invest Dermatol* 1974; **62:** 37). If these effects were to confer some degree of protection against neoplasia, it might be expected that acne would be less common in people who have developed malignant disease.

Seventy patients with lymphomas and leukaemias and one hundred controls were interviewed. Details of past acne vulgaris were recorded and clinical examination performed. A history of acne was equally common amongst patients and controls (57%). However, prescribed therapy for acne was received by fewer patients (4·3%) than controls (15%) ($P<0{\cdot}05$). Examination revealed fewer patients with acne (31·1%) than controls (50%) ($P<0{\cdot}005$). These results suggest a reduced incidence of acne in patients who have developed lymphomas and leukaemias and provide some support for the hypothesis that acne could confer protection against malignancy.

Epidermal growth factor receptor expression in human epidermal tumours. G.R.SHARPE, C.FISHER AND *B.ANGUS, *Departments of Dermatology and *Pathology, University of Newcastle upon Tyne, U.K.*

Normal basal and suprabasal layers of epidermis have high levels of epidermal growth factor receptor (EGFR) expression. In some human carcinomas (breast and bladder) EGFR expression has been shown to correlate with invasiveness and malignancy. The aim of this study was to investigate the relationship between EGFR expression and malignant potential of human epidermal tumours.

Fifty tumours (squamous carcinoma (SC) 2, keratocanthoma (KA) 3, basal cell carcinoma (BCC) 25, Bowen's disease (BD) 3, actinic keratosis (AK) 2, extra-mammary Paget's disease (PD) 2, trichoepithelioma (TE) 2, clear cell acanthoma (CCA) 1, seborrhoeic wart (SW) 6, and viral wart (VW) 4) were studied using standard immunoperoxidase methods. Frozen sections (5 μm) were acetone fixed and incubated with EGFR antibody (EGFR1 10 μg/ml). An indirect peroxidase technique was used with diaminobenzidine as the chromogen. Sections were scored by two independent observers on a five point scale: no staining 0, trace 1, weakly positive 2, positive 3, strongly positive 4. Normal tissue was available from 40 cases and stained for comparison; of these the basal layer was scored as 3 in 70% and 4 in 30%.

Of the tumour types examined SC, KA and SW all scored 4 and consistently showed more EGFR expression than normal tissue. BD, AK, TE and VW scored 3 or 4 with staining at least equal to normal basal epidermis. Four BCCs were graded equal with the corresponding normal tissue, but 20 showed less EGFR expression. The mean score for BCC was 1·75. The CCA scored 1 but only the PD cells scored 0 within a normally staining epidermis. These results show that in skin tumours EGFR expression is not correlated directly with malignant potential. However, squamous differentiation of tumours, both benign and malignant, was associated with increased EGFR expression in the dividing layers prior to keratinization.

Retinoic acid-induced changes in the charge density of murine melanoma cell glycosaminoglycans. M.EDWARD, J.A.GOLD AND R.M.MACKIE, *Department of Dermatology, University of Glasgow, Glasgow, U.K.*

Retinoic acid alters the growth, morphology and differentiation of metastatic melanoma cells grown in culture. These observed changes may be partly facilitated by alterations in the properties of cell surface macromolecules such as glycosaminoglycans which are known to be involved in cell–cell and cell–substrate interactions. We have previously demonstrated that retinoic acid treated melanoma cell glycosaminoglycans exhibit an altered elution profile from anion-exchange columns. We have now examined the effect of retinoic acid on the properties of the individual glycosaminoglycans isolated from B16BL6 murine melanoma cells.

Cells were grown in the presence or absence of 10^{-6} M retinoic acid, and radiolabelled with either $[^3H]$ glucosamine (control) or $[^{14}C]$ glucosamine (retinoic acid-treated) for 48 h. The heat-inactivated medium, trypsin, and cell fractions from the ^3H-labelled cells were combined with the corresponding fractions from the ^{14}C-labelled cells and digested with pronase, dialysed, and the glycosaminoglycans separated on an anion-exchange column. The sulphated glycosaminoglycan peaks were pooled, split into two and treated with either chondroitinase ABC, or nitrous acid, dialysed, and rechromatographed on the anion-exchange column.

The chondroitin sulphates from the medium fractions eluted as two peaks, with the more tightly bound peak from the retinoic acid-treated cells eluting at a lower salt concentration than that from the control cells, while the opposite was observed for the heparan sulphates. The trypsin fractions also showed a reduced binding for retinoic acid-treated cells chondroitins, but no significant difference in the heparan sulphates.

The observed changes in the apparent charge densities of the glycosaminoglycans following retinoic acid treatment, i.e. an increase in heparans and a decrease in chondroitins, may contribute to the altered cellular morphology, growth and differentiation.

Glycosylation of a vitamin A-regulated keratin (cytokeratin 13) in cultured human keratinocytes and A431 cells. IAN A. KING *M.R.C. Clinical Research Centre, Watford Road, Harrow, Middlesex, U.K.*

Retinoids are known to have a marked effect on the cytokeratin pattern expressed by human keratinocytes cultured on 3T3 feeder layers. Here we present evidence that one of these vitamin A-regulated keratins (cytokeratin 13) is glycosylated.

When human foreskin keratinocytes that had been grown in normal tissue culture medium were labelled with $[^3H]$ glucosamine in the presence of tunicamycin they incorporated radioactivity into an acidic 53 kd polypeptide. This component was barely detected in vitamin A-depleted cultures grown in the presence of delipidized serum but was re-expressed when 10^{-7} M all-trans-retinoic acid was added to the culture medium. This $[^3H]$ glucosamine-labelled polypeptide was identified as cytokeratin 13 since it was quantitatively recovered in cytoskeletal preparations from keratinocyte cultures and it was recognized by monoclonal antibodies specific for this cytokeratin in immunoblots following one and two-dimensional gel electrophoresis.

Glycosylation of cytokeratin 13 was also observed in epithelial tumour cell lines and was therefore not peculiar to cultured human keratinocytes. In A431 cells cytokeratin 13 was found to be the only cytoskeleton-associated component labelled with $[^3H]$ glucosamine in the presence of tunicamycin.

The results suggest that cytokeratin 13 is glycosylated at least in cultured epithelial cells. It is not clear why only one out of nineteen cytokeratins should be modified in this way. However, glycosylation is likely to have some significance for the function of this component in the cytoskeleton of epithelial cells.

Fish oil blocks isotretinoin hypertriglyceridaemia but does not reduce low density lipoprotein cholesterol or apoprotein B. J.R.MARSDEN AND C.MARENAH*, *Departments of Dermatology, University Hospital and Clinical Chemistry*, City Hospital, Nottingham, U.K.*

Dietary supplementation with fish oil (MaxEPA) may reduce retinoid hyperlipidaemia (Marsden, *Hum Toxicol*, 1987; **6:** 219). To confirm and extend these findings we have investigated 27 patients (17 male, 8 female aged 18–42 years) with acne. Group 1 ($n = 8$) received 1 mg/kg/day isotretinoin for 8 weeks, group 2 ($n = 8$) received 15 ml/day MaxEPA for 16 weeks and 1 mg/kg/day isotretinoin for weeks 5–12, group 3 ($n = 6$) received 15 ml/day placebo oil for 16 weeks and 1 mg/kg/day isotretinoin for weeks 5–12 and group 4 took 15 ml/day MaxEPA for 16 weeks. Levels of serum triglyceride, cholesterol, low density lipoprotein cholesterol (LDL-C), apoprotein B(apo-B), high density lipoprotein cholesterol (HDL-C), HDL2-C and apo-A1 were measured before treatment and at 2-weekly intervals. Responses were measured by the area under the serum concentration–time curve (AUC) for 0–8 weeks in group 1 and compared with AUC 5–12 weeks in groups 2, 3 and 4. Triglyceride AUC was 34 ± 13 mmol/l week in group 1, 0·1 ± 4 in group 2 ($P < 0{\cdot}05$) and 24 ± 8 in group 3 (N.S.); and − 13 ± 5 in group 4 ($P < 0{\cdot}01$). Cholesterol AUC was 38 mmol/l week ± 14 in group 1, 23 ± 10 in group 2 (N.S.), 28 ± 7 in group 3 (N.S.); and 10 ± 5 in group 4 ($P<0{\cdot}05$). Similarly, LDL-C was 16 ± 6 mmol/l week in group 1, 15 ± 6 in group 2 (N.S.) and 13 ± 3 in group 3 (N.S.); and was 4 ± 2 in group 4 ($P<0{\cdot}05$). Likewise, apo-B AUC was 0·3 g/l week ± 0·1 in group 1, 0·4 ± 0·1 in group 2 (N.S.) and 0·3 ± 0·1 in group 3 (NS.); and 0·1 ± 0·1 in group 4 ($P<0{\cdot}05$). Decreases in total HDL-C were −4 mmol/l week in groups 1, 2 and 3; HDL-C AUC was 5 ± 2 in group 4 ($P<0{\cdot}01$). However, HDL2-C AUC was −6 mmol/l week ± 2 in group 1, −3 ± 2 in group 2 ($P<0{\cdot}05$), and −5 ± 3 in group 3 (N.S.); and 7 ± 3 in group 4. Apo-A1 AUC was −0·03 g/l week ± 0·02 in group 1, −0·30 ± 0·10 in group 2 ($P<0{\cdot}05$), −0·05 ± 0·02 in group 3 (N.S.). We conclude that MaxEPA inhibits isotretinoin hypertriglyceridaemia but does not affect the increase in LDL-C or apo-B; this is probably due to triglyceride depletion of very low density lipoprotein. MaxEPA reduces the decrease in HDL2-C due to isotretinoin but not the fall in total HDL-C; the accompanying reduction in apo-A1 suggests that this may be partly due to decreased HDL particle number as well as reduced cholesterol content.

The effect of isotretinoin on apocrine glands. J.F.B.NORRIS AND W.J.CUNLIFFE. *Department of Dermatology. The General Infirmary at Leeds, Leeds, U.K.*

Isotretinoin produces striking reductions in the size of sebaceous glands and the resulting reduction of sebum production is its presumed mode of action in acne vulgaris. Because acne and hidradenitis suppurativa are occasionally associated, a therapeutic parallel has been drawn between the two conditions, and isotretinoin is now cited as first-line treatment for hidradenitis suppurativa in a major theraputic text (Shelley & Shelley, *Advanced Dermatological Therapy*, W.B. Saunders, 1987, p. 249), although success rates with this drug reported in the literature are variable. The pathogenesis of hidradenitis suppurativa centres upon the apocrine gland, but little is known about the effect of isotretinoin on this structure in either health or disease.

We studied 8 male patients undergoing treatment of acne with isotretinoin at a dose of 1 mg/kg for 4 months. Axillary biopsies were taken immediately pre- and post-therapy and photomicrographs taken of representative sections from each biopsy. Gland size was determined by measuring the areas within individual lumena using a Magiscanner. The areas for each pair of pre and post treatment samples were then compared.

The mean number of lumena ± SD measured in each biopsy was 31·6 ± 8·8 and there was no significant difference between pre- and post-treatment areas in the individual patients ($P>0{\cdot}1$ in 6 patients, $P>0{\cdot}05$ in 2 patients). Equally, there was no significant difference between the pre- and post-treatment areas when the data were pooled ($P>0{\cdot}1$). There were no obvious morphological abnormalities visible with the light microscope.

It would appear, therefore, that isotretinoin does not have the same structural effect on the apocrine gland as it does on the sebaceous gland. The mechanism of action of isotretinoin in hidradenitis suppurativa remains uncertain, but may relate to other properties of the drug such as its anti-inflammatory effect or its ability to reduce ductal hypercornification.

Investigation of the mode of action of cyclosporin A in psoriasis. S.MACNEIL*, R.A.DAWSON*, G.CROCKER*, A.AL ANI†, S.S.BLEEHEN†, G.BOURKE‡ AND D.TIERNEY‡ **Department of Medicine. Clinical Sciences Centre, Northern General Hospital, Sheffield, †Department of Dermatology. Royal Hallamshire Hospital, Sheffield, U.K., and ‡Stiefel Laboratories Ltd., Sligo, Ireland.*

The effectiveness of cyclosporin A in psoriasis opens up new avenues of exploration for the aetiology of psoriasis. In its use as an immunosuppressant, cyclosporin is thought to act on the T lymphocyte, but the involvement of the immune system in psoriasis is far from established. Alternatively, it has been reported that cyclosporin A can act as a calmodulin antagonist *in vitro*. Calmodulin levels are elevated in psoriasis and appear linked to the state of proliferation of the epidermis. Topical application of a calmodulin antagonist has been reported to improve psoriasis. However, cyclosporin A applied topically has not been found to improve psoriasis nor does it inhibit the growth of human keratinocytes in culture.

We have examined the ability of cyclosporin A (Sandimmun®) to inhibit calmodulin activity *in vitro* and to affect proliferation of human keratinocytes and human dermal fibroblasts in culture. We were unable to find any inhibitory effect of cyclosporin A on keratinocyte or fibroblast proliferation or on calmodulin activity *in vivo* at concentrations up to 10 μM. This concentration is 100 times greater than that previously reported to inhibit calmodulin activity *in vitro* and at least 20 times greater than that found in the circulation of patients receiving Sandimmun® for either immunosuppression or treatment of psoriasis.

Thus, we can find no evidence from these *in vitro* studies to substantiate the hypothesis that Sandimmun® may be acting as a calmodulin antagonist. This and the other evidence from the literature failing to find a response to topical application of Sandimmun® suggests that the alternative hypothesis of cyclosporin acting as an immunosuppressant should be pursued more vigorously.

Basement membrane changes in melanocytic tumours.

DANAIDH CLELLAND, RONA M. MACKIE AND CHRISTINE J. SKERROW, *Department of Dermatology, University of Glasgow, Glasgow, U.K.*

The basement membrane (BM) is widely regarded as an essential barrier to tumour cell invasion. There is some evidence that loss of BM components is characteristic of invasive tumours and may be a useful diagnostic indicator of metastatic potential. We have investigated, therefore, the possible correlation between BM alterations and malignancy in 108 human melanocytic tumours, ranging from benign naevi to malignant melanoma. Antibodies to BM components type IV collagen and laminin were used to stain formalin-fixed, paraffin-embedded tissue sections. Binding of antibodies was detected by the PAP technique.

Half of the 6 metastatic melanomas studied exhibited no staining of the BM. The remainder, however, displayed BM staining in at least 50% of their section areas, around both single and nested cells. In all 29 cases of primary melanoma, BM reactivity was lost adjacent to proliferating melanocytes at the dermo-epidermal junction but regained around maturing tumour cells deeper in the dermis. Dysplastic naevi (20 patients), which have been described as occupying a midway position between benign and malignant lesions, presented a pattern of staining very similar to that in primary melanoma. All 16 cases of benign junctional melanocytic naevus also exhibited a reduction or loss of BM staining around junctional melanocytes, with subsequent reappearance around dermal melanocytes as these lesions evolved into benign compound naevi (22 patients) and intradermal naevi (15 patients).

These results show that a similar pattern of distribution of BM components is seen in both benign and maligant melanocytic lesions, and that even in metastatic melanoma BM is not always lost. Alterations in these components should not, therefore, be considered as diagnostic criteria of malignancy in tumours of melanocytic origin.

Endogenous diacyglycerols in normal and psoriatic skin: a mass spectrometric investigation.

C.A.LAUGHLIN-FLYNN AND A.I.MALLET. *Professorial Unit, Institute of Dermatology, U.M.D.S., St. Thomas's Hospital, London, U.K.*

An early event in the activation of inflammatory cells is thought to involve the release of diacylglycerols (DAG) from corresponding phosphoinositols (Michell, *Nature*, 1986; **319:** 176). Synthetic DAGs, especially di-octylglycerol, have been widely reported to mimic the effects of phorbol esters in promoting cell proliferation and the activation of protein kinase C (PKC) in intact cells (Ebeling *et al.*, *Proc Natl Acad Sci USA.*, 1985; **82:** 815). It has been suggested that DAGs play a role in down-regulating PKC in psoriatic lesions (Fisher *et al.*, *J Invest Dermatol*, 1987; **88:** 488(abs)).

We have extracted and identified a number of endogenous DAGs from normal and psoriatic skin in order to verify their existence and to compare the molecular species present. Psoriatic scale and heel callus were homogenized in heptane:propan-2-ol, separated into lipid classes on amino-propyl bonded phase columns and thin layer chromatography. The 1,2-DAGs were derivatized to the *tert*-butyldimethylsilyl ethers and identified by gas chromatography–mass spectrometry by reference to known synthetic standards.

The predominant DAGs present contained hexadecyl and octadecyl acyl chains with varying degrees of unsaturation and no significant difference in the percentage relative intensities of these molecular species could be observed between the two samples. No evidence was seen for the presence of DAGs with other length fatty acid acyl chains. Whether these DAGs can activate PKC remains to be established.

Psoriatic fibroblasts fail to induce epidermal hyperplasia.

W.R.OTTO AND R.DOVER, *Department of Histopathology, Royal Postgraduate Medical School, Hammersmith Hospital, London, U.K.*

The role of the dermal component may be important in the development of the psoriatic lesion (Saiag *et al.*, *Science* 1985; **230:** 669). These workers embedded full thickness biopsies from psoriatic skin into collagen gels seeded with normal (NF) or psoriatic (PF) fibroblasts, and obtained increased keratinocyte outgrowth from the latter. However, no control was made for the influence of the biopsy dermal remnant, nor was a histological analysis made. We compared the ability of NF or PF to influence the behaviour and histology of keratinocytes when grown together in an *in vivo* assay. Adult dermal fibroblasts from age- and sex-matched normal or psoriatic involved skin were grown using the methods of Rheinwald & Green (*Cell* 1975; **6:** 331). Second passage keratinocytes were mixed with seventh passage fibroblasts at a ratio of 10:1 and were injected intradermally into nude mice at 2×10^6 cells per site in 0·1 ml culture medium. Cysts were produced which were examined at 3,7,10,28 and 48 days by light and electron microscopy.

All cysts showed increasing organization and differentiation by day 10. Mitoses were confined to the basal cells, a spinous and well developed granular layer were observed, squames were shed into the lumen of the cyst. The peri-epithelial stroma was of human origin as demonstrated by a species specific dye. At no time did PF induce hyperplasia or parakeratosis; indeed, the epithelium looked quite normal. By day 28 the cysts were attacked by host phagocytes, and angiogenesis into the cysts occurred until day 48.

We conclude that: (i) the ability of NF or PF to influence the differentiation of normal keratinocytes was similar; (ii) other factors are involved in the epidermal dysfunction in psoriasis than an epithelial-mesenchymal interaction; (iii) these factors may reside in other cellular or humoural agents and their interactions; (iv) this model may prove useful in the search for such factors.

A new method for studying *in vivo* leukocyte emigration in man.

A.J.RUTMAN, C.E.M.GRIFFITHS, I.E.ADDISON*, B.JOHNSON* AND LIONEL FRY. *Department of Dermatology, St. Mary's Hospital, London W2, and *Department of Haematology, University College Hospital, London, U.K.*

The method is based on a skin window technique developed by one of us (Addison *et al.*, *J Immunol Methods* 1982; **54:** 129). In the original technique, abrasion with a slowly rotating dental bur was used both to remove the epidermis and to induce a strong inflammatory response. Our aim in this study was to develop a technique which itself produces only a weak cellular infiltration, thus enabling the effects of exogenous substances on leukocyte emigration to be studied.

Rectangular areas (3 × 6 mm) on the flexor surfaces of the forearm or upper arm of ten healthy volunteers were subjected to epidermal tape-stripping. Two cellulose nitrate membranes were placed over each site and overlaid with a filter pad soaked in attractant (fresh plasma, zymosan-activated plasma or 10^{-7} M F-Met-Leu-Phe) or saline. The membranes were removed after periods of 1–12 h, stained and leukocyte emigration assessed by counting the number of foci of infiltrating cells. In four individuals, 3 mm punch biopsies of stripped sites were taken and processed for routine histology.

The tape-stripping produced little or no discomfort. Histological examination revealed that the stripping had removed the stratum corneum but the stratum granulosum remained intact. Also, vertical clefts extending from mid-dermis to epidermis were seen in most sections. At 90 min, in the absence of attractant, there was only a mild, early inflammatory response, whilst 90 min after the application of plasma or F-Met-Leu-Phe, there was marked oedema and a mainly granulocytic infiltration extending to the skin surface. The following foci counts were obtained for F-Met-Leu-Phe membranes at three time points (saline control values in brackets): 2 h: 20 (5), 3 h 36·5 (8·5), 6 h 400 (54·5).

Our findings show that tape-stripping is a reliable means of removing the keratinized barrier to emigration without itself producing a significant inflammatory response. Many such skin windows can be performed on the same individual with minimal discomfort. This is therefore a useful model for studying the *in vivo* cellular response to putative inflammatory mediators as well as for assessing the effects of anti-inflammatory drugs on this process.

Enhanced expression of basement membrane zone antigens in psoriatic skin. C.J.M.STEPHENS, L.RUMSBY AND F.WOJNAROWSKA, *Wycombe General Hospital, High Wycombe, Bucks, U.K.*

Disturbance of the basement membrane zone (BMZ) antigens in psoriasis is suggested by the well documented association with bullous pemphigoid (BP) (Grunwald *et al., J Am Acad Dermatol* 1985; **13:** 224) and recent electron-microscopy studies have shown structural discontinuity of the basal lamina and functional abnormalities of the basement membrane zone (Heng *et al., J Invest Dermatol* 1986; **87:** 362). There has been no previous study of the composition of the BMZ in psoriasis.

Eleven patients with psoriasis were studied. Paired punch biopsies from lesional and non-lesional skin from similar anatomical sites were performed. Immunofluorescence was performed using a standard technique, and sera containing circulating antibodies to BP, epidermolysis bullosa acquisita (EBA), adult linear IgA disease (LAD), chronic bullous disease of childhood (CBDC), and cicatricial pemphigoid (CP) antigens were studied, at a dilution that gave a weak positive (±) or negative (−) on normal skin. Laminin, type IV collagen and fibronectin were also studied.

There was enhanced expression of the antigens and laminin in uninvolved psoriatic skin (+ +) and greatly enhanced expression (+ + +) BP 8/11, CP 5/11, EBA 7/11, LAD 11/11, CBDC 10/11 in lesional skin. The LAD sera bound to the whole surface of the basal cells in psoriatic skin. There was also enhancement of laminin (7/11). Fibronectin and type IV collagen did not differ from normal skin.

This enhanced and altered expression of BMZ components in psoriasis may be related to the basal keratinocyte hyperproliferation or lamina densa reduplication that occurs in this disorder. Alternatively, BMZ components may be unmasked by the action of proteolytic enzymes from granulocyte or T cell interactions.

Differentiation of psoriatic and normal keratinocytes in organotypical and keratinocyte culture as assessed by monoclonal antibodies to keratins. H.A.NAVSARIA, I.C.MACKENZIE AND I.M.LEIGH. *Experimental Dermatology Laboratory, London Hospital, London, U.K.*

Monoclonal antibodies to individual keratin polypeptides can be used to study epithelial differentiation as keratin expression varies in a tissue-specific way. In epidermis, keratin expression alters as the cell moves through differentiation. Epidermal keratinocytes can be grown *in vitro* under conditions which modulate differentiation on plastic substrate with a feeder layer (Rheinwald & Green, *Cell* 1975; **6:** 331) or in organotypical culture (Mackenzie & Fusenig, *J Invest Dermatol.* 1983; **81:** 189).

Keratinocytes from neonatal foreskin, adult abdominal skin and untreated plaque psoriasis were cultured in both Rheinwald Green and organotypical systems and examined using a large panel of well characterized monoclonal antibodies to single keratin polypeptides. Cultured keratinocytes from neonatal foreskin expressed the mucosal keratins 4 and 13 (Moll *et al., J Cell Biol* 1982; **95:** 285) in addition to the epidermal keratins 1/10 and the hyperproliferative keratins (6/16) suprabasally when cultured on plastic but not in organotypical culture. All keratinocytes expressed keratin 19 on plastic but not in organotypical culture. All cultures expressed keratins 6/16 suprabasally, i.e. the hyperproliferative keratins.

Thus, *in vitro* no difference was found in keratin expression between psoriatic and normal keratinocytes but all cultures expressed the hyperproliferative keratins 6/16 suprabasally so that 'normal' keratinocytes expressed the psoriatic 'hyperproliferative' profile. Foreskin keratinocytes could also express the mucosal keratins 4/13 in Rheinwald Green cultures.

Alteration of lectin binding to cultured human keratinocytes by extracellular calcium. A.M.AL-ANI, A.G.MESSENGER, S. MACNEIL* AND S.S.BLEEHEN, *Departments of Dermatology and *Medicine, University of Sheffield, U.K.*

Human keratinocyte (HK) proliferation and morphology can be modulated by changing extracellular calcium. In this study we have examined whether such changes represent changes in differentiation in these cells by examining lectin binding to cell surface sugars.

Lectin binding to cultured HK was studied in normal calcium medium, 1·15 mmol/ml (NCM) and low calcium medium, 0·15 mmol/ml (LCM), using 6 different biotinylated lectins and fluorescent avidin. (All lectins treated with their inhibitory sugars gave negative staining).

In NCM all the lectins tested gave positive surface binding on the keratinocytes, especially at points of contact. The binding was found in all the cell layers. However, in LCM there was complete absence of membrane binding of all lectins and there was intracellular perinuclear staining. On switching HK from the LCM to NCM, there was a return of the surface staining which was noted with the return of stratification and desmosomes.

These results show that low calcium can reversibly alter the lectin binding pattern to cultured HK, affecting glycoprotein and glycolipid synthesis as well as cell-to-cell contact. Thus, calcium can modulate cell differentiation in HK and may prove a convenient approach to the study of agents which affect differentiation of HK.

Rates of DNA and protein synthesis in freshly isolated rat hair follicles and after 7 days organ maintenance. M.Philpott and T.Kealey, *Nuffield Department of Clinical Biochemistry, University of Oxford, John Radcliffe Hospital, Headington, Oxford, U.K.*

The isolation of viable hair follicles may provide a useful model for alopecia. We report on rates of DNA and protein synthesis in rat hair follicles isolated by the shear method of Green *et al.* (*J Invest Dermatol* 1986; **87:** 768) and show how such activities survive 7 days organ maintainance. Rates of DNA and protein synthesis were studied by measuring incorporation of $[^3H]$ thymidine (specific activity 91 μCi/nmol) and $[U\text{-}^{14}C]$ leucine (specific activity 2 μCi/μmol), respectively, into PCA precipitable material. Time courses (0, 1, 3, 6 h) showed uptake to be linear, 20 follicles in duplicate were used for each time-point.

Rates of thymidine and leucine uptake for follicles from 8 day old rats were 1·98 ± 0·29 fmol/μg DNA/h (mean ± SEM, $n = 4$) and 15·1 ± 1·94 pmol/μg DNA/h ($n = 4$), respectively. Rates of uptake for follicles maintained for 7 days on polycarbonate filters (0·45 μm) on supplemented Williams E medium were 1·64 ± 0·19 fmol/μg DNA/h ($n = 4$) for thymidine and 28·6 ± 0·4 pmol/μg DNA/h ($n = 4$) for leucine. Because, in rats, hair growth is cyclical, rates of uptake were studied for litter mates at 15 days. Rates of thymidine uptake were 2·05 ± 0·13 fmol/μg DNA/h ($n = 4$) and for leucine 23·72 ± 2·46 pmol/μg DNA/h ($n = 4$).

Rates of uptake for follicles from 10 day old rats were 3·05 ± 0·46 fmol/μg DNA/h ($n = 4$) for thymidine and 105 ± 17·5 pmol/μg DNA/h for leucine. After 7 days organ maintainance thymidine uptake was 2·06 fmol/μg DNA/h and leucine 32·3 ± 5·58 pmol/μg DNA/h. Fresh follicles from 17 day rats gave values of 0·78 ± 0·18 fmol/μg DNA/h for thymidine and 15·9 ± 1·65 pmol/μg DNA/h for leucine.

These results indicate that our biochemical observations on freshly isolated and maintained follicles may eventually provide a useful model for human alopecia. However, microscopic observations on maintained follicles indicate premature keratinization of the bulb, and autoradiographic studies will demonstrate the pattern of cell division.

Release of heparin-related glycosaminoglycans and histamine from human basophils by house dust mite in atopic patients. K.M.Reilly, J.Dawes, P.L.Yap, I.R.MacGregor and R.StC.Barnetson, *M.R.C./S.N.B.T.S. Components Assay Group, Edinburgh, South of Scotland Blood Transfusion Service & Department of Dermatology, University of Edinburgh, U.K.*

The measurement of histamine release from basophils has been extensively used for *in vitro* studies of human allergy; heparin related glycosaminoglycans (GAG) is also released from the granules of basophils in allergic subjects, but this has received little attention previously.

Fourteen atopic subjects were studied, ten patients with atopic eczema and four atopic volunteers; all had a RAST score of 4 for house dust mite allergen. They were compared with 8 non-atopic controls who had a RAST score of 0 for this allergen. Assay of histamine on the patients' leukocytes was carried out following basophil release induced by house dust mite, using a fluorimetric method. Assay of GAG was performed by using the radio-assay developed by Dawes *et al.* (*Thromb Haemost* 1985; **54:** 630).

GAG was found to be contained only in the basophil fraction of whole blood and its release, like that of histamine, was calcium-dependent. It was released from the basophils of atopic individuals in a dose–response pattern after allergen challenge, and from non-atopic individuals in smaller quantities with no obvious dose dependence. Histamine was only released from cells of atopic individuals after allergen challenge and with a different dose response from that observed for GAG release. Moreover, the release of GAG occurred with a different time course from the release of histamine from basophils *in vitro*. This may be explained by the theory of Uvnas (*J Invest Dermatol* 1978; **71:** 76) which postulates the formation of a pore, which acts as a dialysing membrane, when fusion between the secretory granule membrane and plasma membrane occurs at degranulation.

Livedo reticularis, anticardiolipin antibodies, CNS complications and tissue plasminogen activator levels. S.C.MAYOU, R.A.ASHERSON*, M.M.BLACK, D.A.P.COOKE† AND G.R.V.HUGHES*. *Department of Dermatology, *The Lupus Arthritis Research Unit and †Surgical Unit, St. Thomas' Hospital, London, U.K.*

The association of livedo reticularis (LR) with cerebrovascular accidents is documented as Sneddon's syndrome. A high frequency of LR among SLE patients with the antiphospholipid syndrome has also been noted. A study to determine the relationship of LR in SLE with anticardiolipin antibodies (aCL) to CNS symptoms and to investigate non-livedo for the presence of aCL was carried out.

Impaired production of tissue plasminogen activator (tPA) occurs in atrophie blanche (Pizzo *et al., Arch Path Lab Med* 1986; **110:** 517). LR may result from endothelial cell damage and stasis of superficial blood flow. Therefore, tPA in involved and non-involved skin was determined.

Sixty-three patients with LR were examined for CNS symptoms. The aCL and tPA were measured by an ELISA method. Thirty two patients were aCL positive and 31 negative. Fourteen of 20 (44%) of the aCL positive had CVAs or TIAs whereas only 2 of 31 (7%) of the aCL negative group were affected. Epilepsy, chorea, psychiatric symptoms or dementia occurred in 21 of 32 (66%) of the aCL positive group but only 1 of 31 (3%) of the aCL negative group. Twenty patients with SLE were aCL positive with CNS manifestations in 14 of 20. In 11 aCL negative patients and 20 aCL negative idiopathic, mixed CTD, vasculitic and vasospecific associated LR, CNS symptoms did not occur. The most florid and extensive LR occurred in the aCL positive SLE group. tPA levels in normal skin were greater (3–6·5 IU/ml) than in involved skin (< 0·5–4·9 IU/ml).

The association of aCL and LR in SLE is strongly associated with CNS complications. SLE and LR unassociated with aCL does not have this association. tPA levels are reduced in livedo skin suggesting a possible aetiological mechanism.

A sequential histopathological study of dermographism. J.S.C.ENGLISH, G.M.MURPHY, R.K.WINKELMANN AND B.BHOGAL, *St. John's Hospital for Diseases of the Skin, 5, Lisle Street, London, U.K.*

The precise mechanisms involved in dermographism are not known. As a prerequisite to subsequent pharmacological studies, the time course of the cell types involved in dermographism has been studied.

Weals were induced in 4 dermographic patients with a standard pressure ($7{\cdot}2 \times 10^5$ Pa). Biopsies were taken from non-whealed skin and from the induced wheals at 5 min, 15 min, 2 h and 24 h. Two further dermographic patients had biopsies taken immediately after induction and at 2 hours from wheals induced by 2 different pressures ($2{\cdot}5 \times 10^5$ and $10{\cdot}9 \times 10^5$ Pa). Additional control skin biopsies were taken from 8 non-dermographic individuals.

The histological changes were seen only in the upper dermis. Non-traumatized skin showed a significantly raised mean mononuclear cell count ± SE ($15{\cdot}6 \pm 2{\cdot}3$ compared with $8{\cdot}1 \pm 0{\cdot}6$ in non-dermographic controls; $P = 0{\cdot}0002$). There was some endothelial cell swelling seen at 5 min which persisted at 24 h. The mononuclear cell infiltrate did not change quantitatively in any observation period; neutrophils were seen at 5 min, reached a peak at 2 h and were still present at 24 h; there was, however, a great inter-patient variation in the degree of neutrophilia. Mast cell numbers did not change significantly during the time course and were numerically similar to those found in non-dermographic individuals. There was no qualitative or quantitative difference in the infiltrate in the wheals induced by the 2 different pressures. Immunofluorescence studies were negative in all the biopsies.

The main histological changes were the increased mononuclear cell infiltrate in non-whealed skin, and the presence of neutrophils and venule wall swelling in whealed skin.

IgG subclasses in bullous pemphigoid, pemphigus and herpes gestationis. R.CERIO, B.S.BHOGAL, S.KELLY AND M.M.BLACK. *Institute of Dermatology, St John's Hospital for Diseases of the Skin, London, U.K.*

Complement activation is thought to play a major role in the pathogenesis of most autoimmune bullous disorders. With the exception of IgG4, all IgG subclasses can bind complement. Employing monoclonal antibodies in both indirect and direct immunofluorescence techniques, we examined the distribution of IgG subclasses in serum and cutaneous tissue from 12 patients with bullous pemphigoid (BP), 10 with pemphigus vulgaris (PV), 2 with pemphigus foliaceus (PF) and in the serum of 24 patients with herpes gestationis (HG). C_3 was present in all the tissues investigated.

Our results confirm earlier reports (Sams & Schur, *J Lab Clin Med* 1972; **82:** 249; Bird *et al.*, *J Invest Dermatol* 1986; **86:** 21), that the IgG subclasses distribution of autoantibodies were heterogeneous in BP, PV and PF. IgG4 was the predominant subclass found in serum and tissue. It was present exclusively in 2 cases of BP and one of PV. IgG1 was isolated from most patients but IgG3 and IgG2 were rarely found. Only 9 of 12 of BP sera fixed complement, including one where only IgG4 was present. In HG, IgG1 was the predominant subclass and was found in all sera analysed. IgG3 was found in 3 sera but IgG2 was not detected. IgG4 was only found in one case.

These findings demonstrate that in BP and pemphigus the non-complement fixing IgG4 autoantibody predominates whereas in HG except for one patient, IgG4 was not present and the circulating autoantibody was IgG1. Furthermore, as C3 was identified in all tissues studied, this suggests that IgG4 may play an important role in the pathogenesis of BP, PV and PF and C3 activation is probably a secondary event. In contrast, complement fixation by IgG1 seems to be the mechanism of tissue injury in HG.

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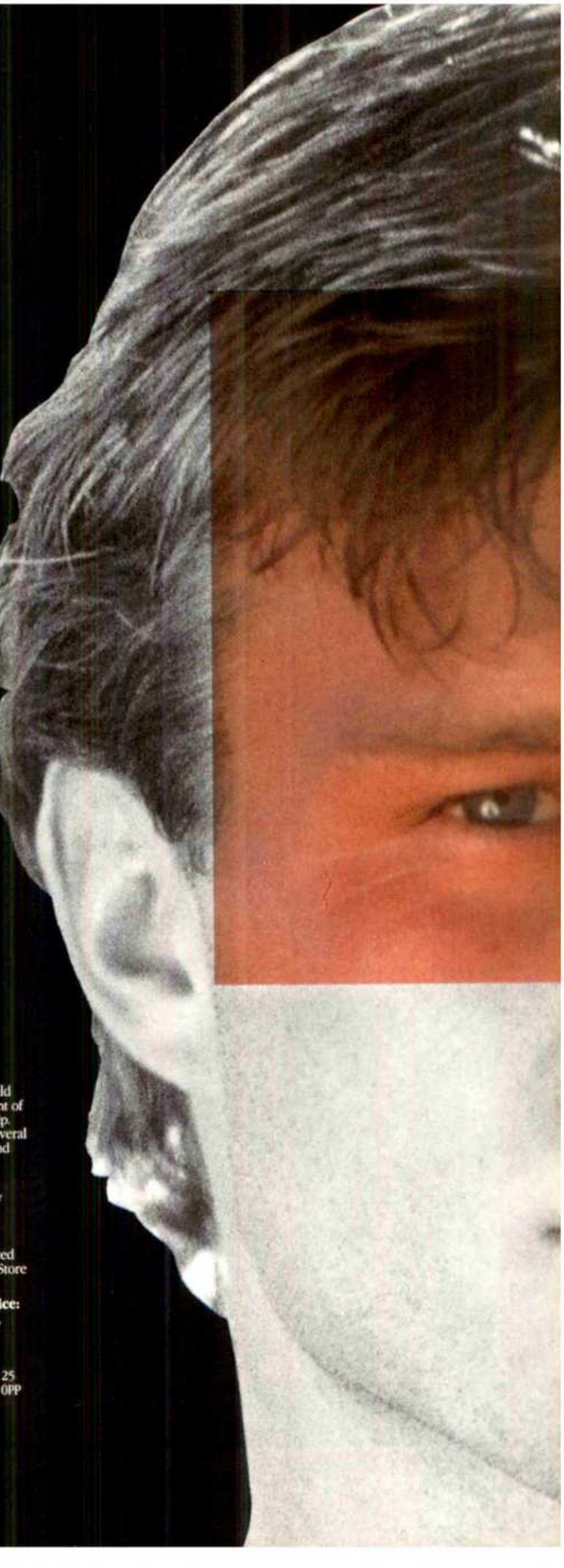

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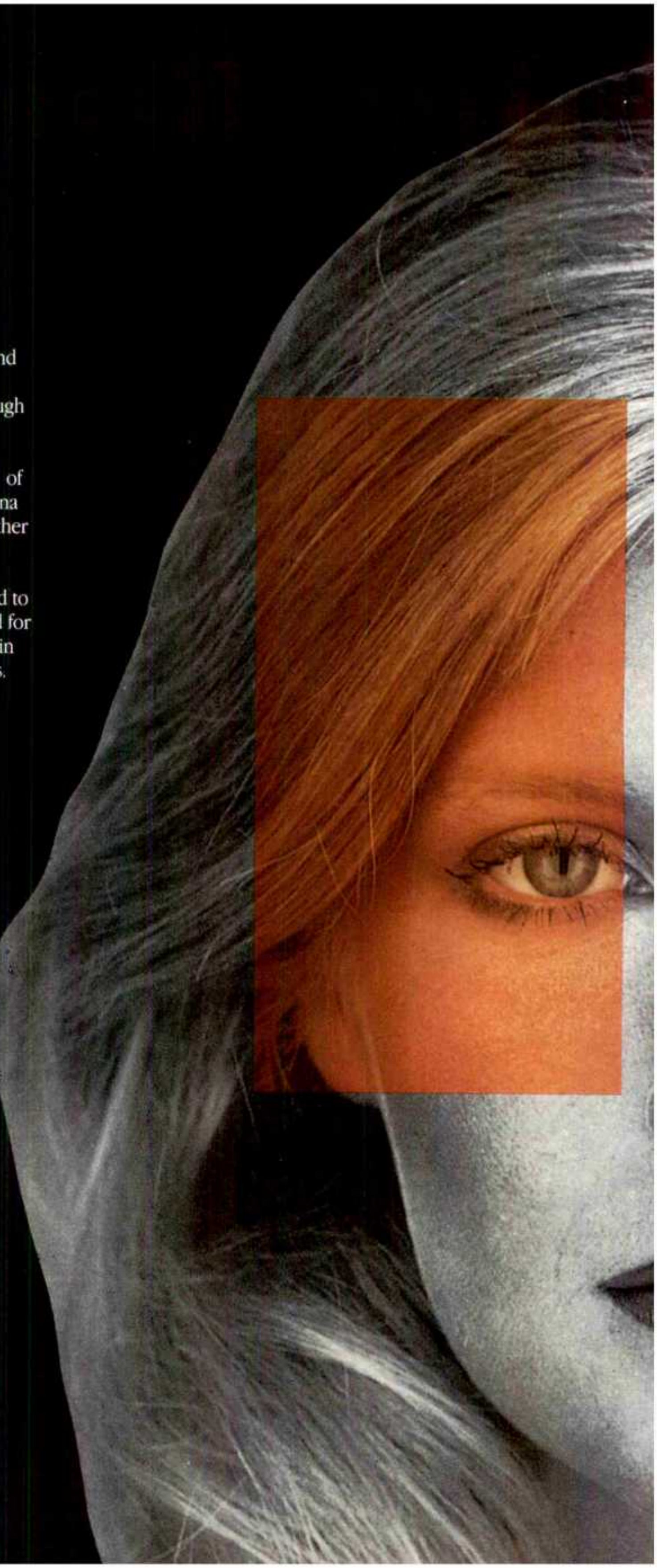

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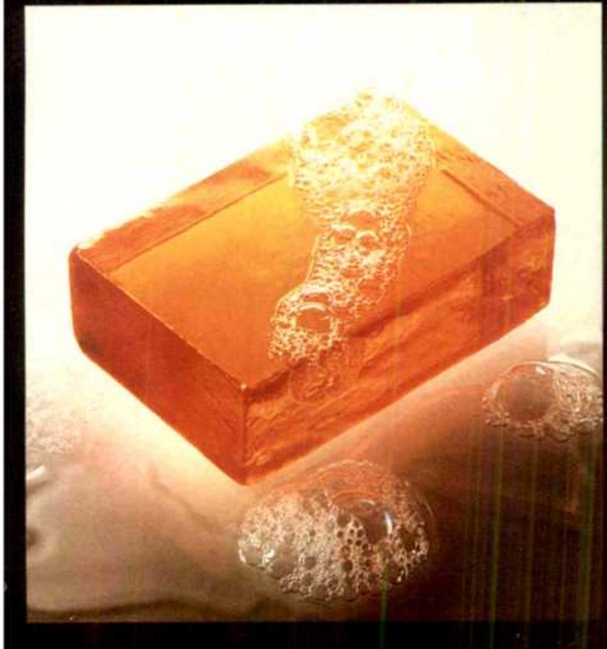

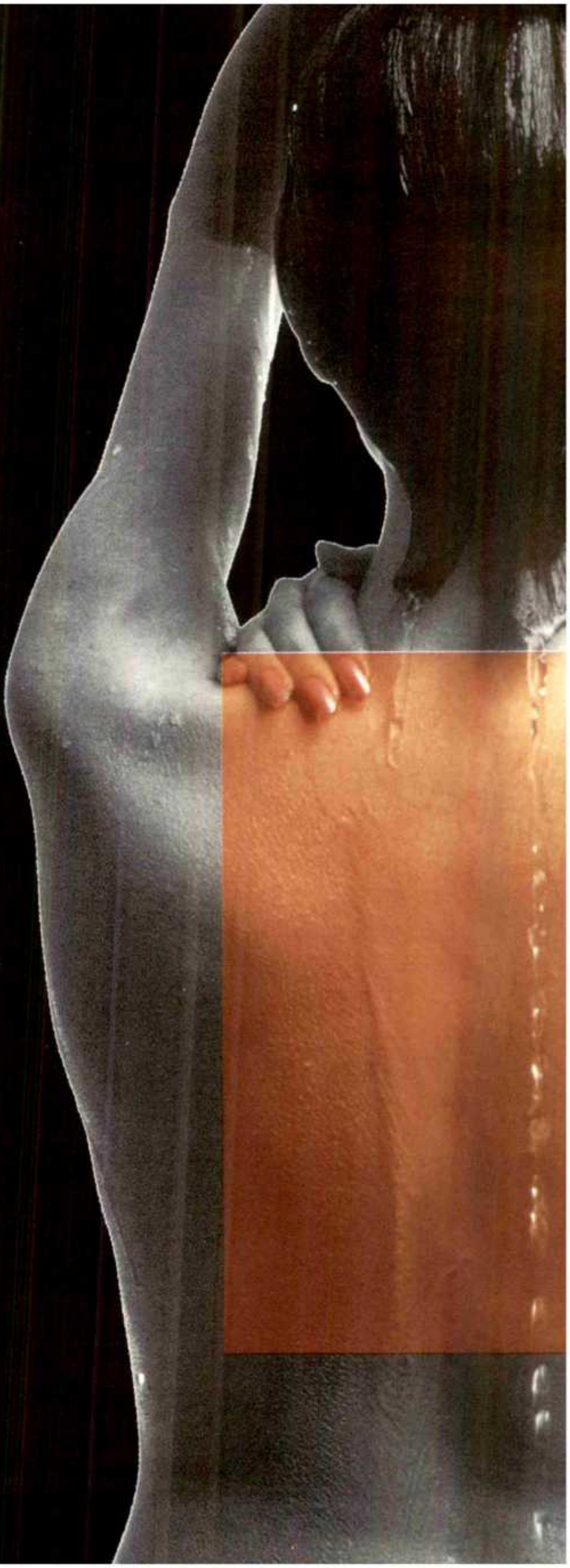

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British Journal of Dermatology (1988) **118,** 303–312.

Correspondence

Pemphigus foliaceus localized to the nose

MADAM, We noted with interest the recent paper entitled 'Tin-tack' sign in localized pemphigus foliaceus (*British Journal of Dermatology*, 1987; **116:** 127), in which two cases of pemphigus were reported, limited to the nose for 18 weeks and 18 months, respectively. We would like to draw your attention to three further cases of this type of localised pemphigus, providing evidence for a recognisable disease sub-group.

Case 1. A 27-year-old black man had an eroded, scaly red plaque of 8 weeks duration on the left side of his nose. The histological appearance was of a sub-corneal bulla with considerable acantholysis (Fig. 1). Direct immunofluorescence (IF) showed intercellular IgG and C_3, and indirect IF was positive. The eruption failed to respond to treatment with tropical Dermovate, but the patient was lost to follow-up at 6 months, at which time no dissemination of the disease had occurred.

Case 2. A 62-year-old woman had a scaly red plaque localized to her nose for 10 months (Fig. 2). Histologically there was acantholysis in the granular cell layer (Fig. 3). Direct IF was positive with intercellular fluorescence for C_3. Indirect IF was also positive at a titre of 1:320. The eruption did not respond to treatment with fluorinated topical steroids, but was controlled using oral cyclophosphamide. Scattered small superficial erosions subsequently occurred on the trunk.

Case 3. A 43-year-old white man presented with a sharply defined red plaque on his nose with associated blistering of 9 years duration. A clinical diagnosis of localized pemphigus was made and the eruption was controlled using oral prednisolone 20 mg/day, although a maintenance dose of 5–15 mg/day was subsequently required to prevent relapse.

Pemphigus foliaceus is generally recognized as a benign although long lasting condition. Scaly red plaques, often sharply delineated, are a common feature,[1] as in our three patients, and localized disease may occur. In Perry and Brunsting's review of 19 cases[1] two cases remained localized (to the scalp and umbilical areas, respectively) for 14 months.

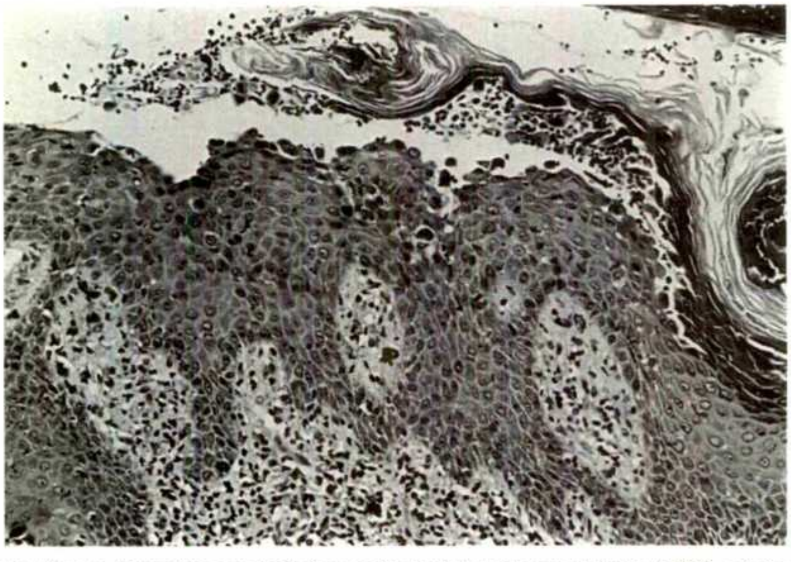

FIGURE 1. Case 1. Extensive acantholysis associated with a subcorneal bulla. (H&E, original × 300).

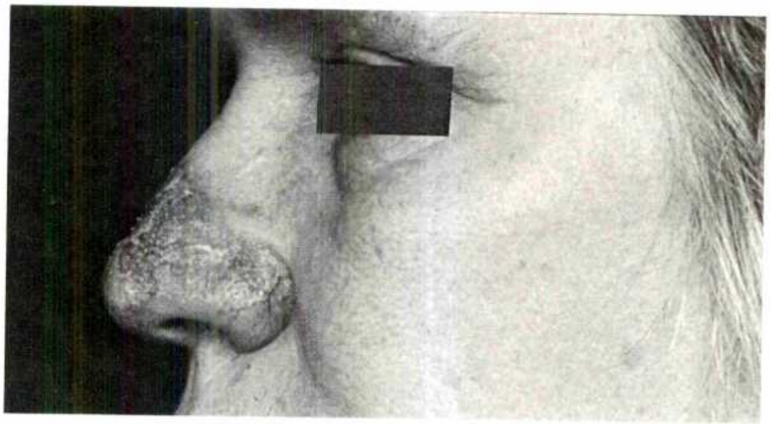

FIGURE 2. Case 2. Scaly red plaque on the nose, of 10 months duration.

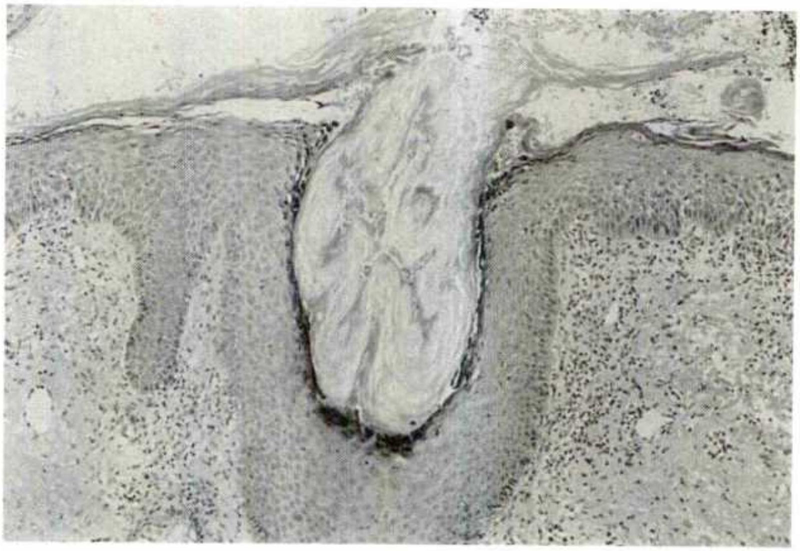

FIGURE 3. Case 2. Acantholysis of the granular cell layer. (H&E. Original × 300).

We recognize this curious pattern of disease localization in pemphigus foliaceus as a particular subgroup of the condition. The absence of basement membrane deposition of immunoglobulin, and negative antinuclear antibody tests makes the diagnosis of pemphigus erythematosus untenable in these cases. The cause of the localization to the nose is obscure. However, ultraviolet (UV) radiation has been used experimentally to induce acantholysis in pemphigus[2] and clinical exacerbation of the disease as a result of exposure to UV is occasionally seen in practice. Thus, it is possible that UV is an important aetiological factor.

Dowling Skin Unit and the Institute of Dermatology,
United Medical Schools of Guy's
and St Thomas's Hospitals, London
*Kings College Hospital, London, U.K.
**General Infirmary, Leeds, U.K.

JULIA A.NEWTON
D.H.MCGIBBON
B.MONK*
N.R.ROWELL**

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Spontaneous improvement of congenital malalignment of the great toe nails

MADAM, The condition now termed congenital malalignment of the great toe nails was first described by Samman[1] under the title great toe nail dystrophy. The essential underlying abnormality is lateral deviation of the long axis of nail growth relative to the distal phalanx.[2] This results in a discoloured, thickened, pointed nail with prominent transverse ridging and an inability to grow to normal length. It has been suggested that corrective surgery should be performed early.[3] We present here two cases where spontaneous improvement occurred by the age of 5 years, which illustrate the fact that even severely dystrophic nails can revert to normal.

Case 1: A 19-month-old boy was seen with a history of dystrophic toe nails since birth. The great toe nails deviated laterally, were ridged and thickened, and failed to grow to normal length (Fig. 1). There was a history of spontaneous shedding of the nails. Surgical treatment was considered, but after discussion the child was managed conservatively with regular review. By the age of 3 years the nails were still obviously malaligned, but were growing to normal length, with less discolouration and ridging. At 5½ years of age, all the nails were virtually normal (Fig. 2).

Case 2: A 20-month-old girl was noted to have dystrophic great toe nails at birth. On examination the nails were malaligned and ridged. By the age of 5 years the nails had become normal.

Baran[3] suggested that congenital malalignment of the big toe nails should be treated surgically before the age of 2 years to prevent later complications such as ingrowing nail and hemionychogryphosis. The operation described realigned the nail bed allowing normal growth.

Dawson[4] described two families with congenital malalignment of the toe nails, inherited in an autosomal dominant fashion. The degree of dystrophy varied among members of the same family. Whilst the changes were most marked in the great toe nails, in some patients the other nails were also abnormal. A follow-up report[5] documents improvement in three of the cases. One of these was severely affected at presentation and improved very markedly. Baran,[6] suggested that the variable prognosis reflected the degree of severity of the condition and recommended early correction in severe cases. Our experience, like Dawson's, suggests that even marked dystrophy can return to normal. In the absence of complications we feel treatment should be conservative.

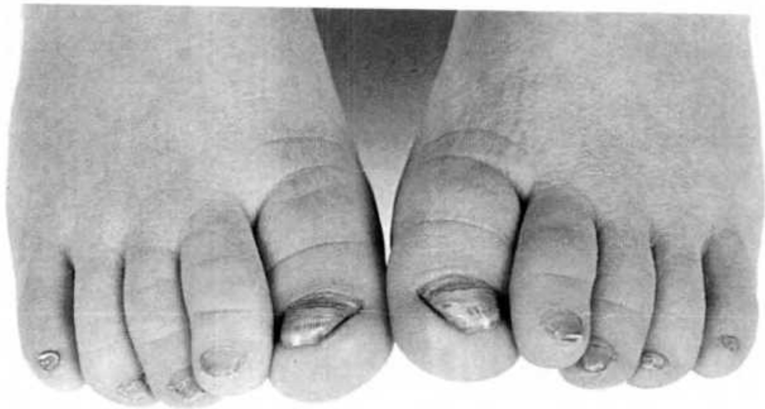

FIGURE 1. Case 1: Aged 19 months.

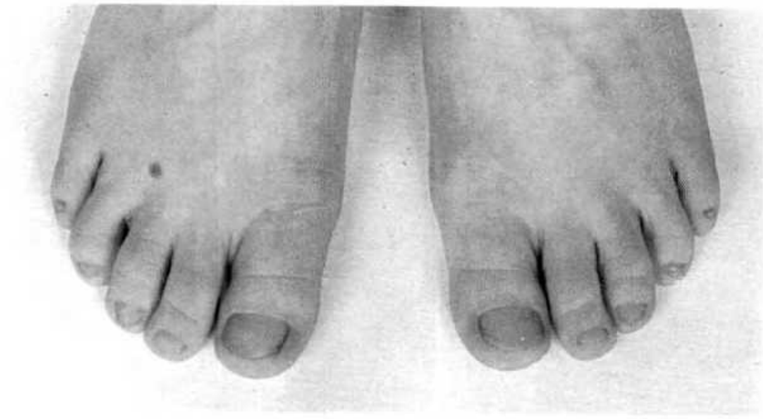

FIGURE 2. Case 1: Aged $5\frac{1}{2}$ years.

Department of Dermatology,
Bristol Royal Infirmary
Bristol BS2 8HW, U.K.

S.E.HANDFIELD-JONES
R.R.M.HARMAN

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I am grateful to Dr C.T.C.Kennedy for allowing me to report Case 2.

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Penicillin-induced pemphigus vulgaris

MADAM, A number of drugs have been implicated in the induction of pemphigus including penicillamine, pyritinol, captopril, rifampicin, propranolol and phenylbutazone.[1] The exact mechanism by which drugs provoke pemphigus, however, remains to be elucidated. A case of pemphigus following long-term penicillin therapy was described by Ruocco *et al.*,[2] but the bullous disorder was attributed to penicillamine rather than the penicillin molecule. This was supported by the finding of penicillamine, a metabolic breakdown product of penicillin, in the serum and an interval of more than 6 months before the onset of symptoms, consistent with other reports of penicillamine-induced pemphigus.[3] Furthermore, taste disturbance, a generalized pruritic macular eruption, eosinophilia and eosinophilic spongiosis, all features documented in penicillamine-induced pemphigus, were also present. We describe a patient who developed pemphigus vulgaris following a course of penicillin, but in whom the typical features associated with penicillamine induced pemphigus were absent.

A 71-year-old Kenyan-Asian man received a 19-day course of intramuscular procaine penicillin 2·4 g daily and oral probenecid 2 g daily for asymptomatic neurosyphilis. He developed multiple, discrete crusted lesions varying from 0·7 cm to 5·0 cm in diameter 1 week later on the scalp, spreading to the trunk and limbs. There were also a few small erosions on the chest, but no bullae. The Nikolsky sign was negative and the oral mucosa was clear. There was no family history of pemphigus or penicillin hypersensitivity and previous exposure to penicillin was denied. A skin biopsy showed the characteristic histological and immunofluorescence features of pemphigus vulgaris. Intercellular cement substance antibody was positive at 1:100. His HLA genotype was A9, B5, B40. Topical treatment with potent corticosteroid ointment resulted in considerable improvement, but new lesions were continuing to erupt 1 year later.

There is striking histological resemblance on electron microscopy between the early changes of pemphigus vulgaris and those of morbilliform eruptions developing as a delayed reaction to penicillin.[4] A circulating anti-epithelial antibody as well as antibody bound to the epidermal intercellular cement substance has been demonstrated in maculopapular penicillin eruptions.[5] Clinical pemphigus does not usually develop with penicillin, probably because the pathogenetic anti-epithelial antibody is present transiently and at a low titre, and the genetic susceptibility to the development of pemphigus is lacking.

In our patient the onset of pemphigus vulgaris soon after penicillin therapy and the success of a topical corticosteroid in controlling the eruption are compatible with the bullous disorder being induced by penicillin. However, in the absence of other well recognized adverse reactions to penicillin and without a challenge test, the possibility that the occurrence of pemphigus vulgaris was coincidental cannot be excluded.

The Skin Hospital,
Birmingham B15 1BR, U.K.

P. DUHRA
I. S. FOULDS

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Acute generalized exanthematous pustular dermatitis induced by diltiazem

MADAM, acute generalized exanthematous pustular dermatitis can be induced by several factors.[1,2] The most widely known type is psoriatic pustular dermatitis. However, increasingly often, exanthematous pustular dermatitis occurs, not necessarily because of a genetic predisposition to psoriasis, but following drug ingestion, or, in some cases, in relation to a viral infection. We report here a case of exanthematous pustular dermatitis following diltiazem therapy.

A 77-year-old woman with a 10-year history of asthma was hospitalized in May 1986 with a febrile eruption which had begun 18 days earlier. A generalized disseminated pustular erythema covered the trunk and limbs. Her temperature was 38°C. There was no mucosal involvement. Physical examination, including palpation for liver and spleen, was otherwise normal. Skin biopsy revealed subcorneal neutrophilic pustules associated with a perivascular lymphocytic infiltrate in the superficial and mid dermis. Direct immunofluorescence showed perivascular deposits of C_3 and IgM in the superficial dermis only.

Laboratory studies indicated severe inflammation with thrombocytosis (540×10^9/l), an elevated white blood cell count ($32{\cdot}6 \times 10^9$/l; 93% neutrophils), hyperfibrinogenemia (7g/l), elevated ESR (80–100 mm/h) and moderate hepatic cytolysis (GOT 35 IU/l, GPT 36 IU/l, GT 160 IU/l). The serum concentrations of calcium (2·32 nmol/l) and phosphate (0·85 nmol/l) were normal. There was no evidence of bacterial or viral infection. The following were normal or negative: circulating immune complexes, cryoglobulins, serum IgE level, and serum vitamin D. For a period of 10 days preceding the eruption the patient had been treated with diltiazem (Tildiem®) and vitamin B12 (Arphos®) and for 2 days before she had taken efloxate (Recordil®).

Before admission, the vitamin B12 and efloxate had been withdrawn without effect. After hospital admission diltiazem was withdrawn and the cutaneous lesions then disappeared within 5 days.

From May to October 1986, she was treated with digoxin (Coragoxine®) and nifedipine (Adalate®). In October 1986, the nifedipine caused oedema of the feet and was replaced by diltiazem. Forty-eight hours later an acute febrile exanthematous and pustular eruption appeared on the knees and buttocks.

Laboratory tests were normal, except for a moderately elevated ESR (60–80 mm/h). There was no significant change in the serum calcium or phosphate levels.

A skin biopsy showed a subcorneal neutrophilic pustule. Direct immunofluorescence showed intravascular deposits of C_3 and C_{1q}. Three days after diltiazem was withdrawn the skin returned to normal. The disappearance of the dermatitis after the withdrawal of diltiazem and its recurrence upon further administration of the drug points to the reaction being caused by the calcium channel blocker.

In general, side effects of diltiazem are rare (4% of patients treated), but can include headaches, vertigo, flushing, dizziness, macular skin eruptions and sweating.[3-5] A case of photosensitive lichen planus was reported by Hashimoto *et al.*[6] An exanthematous pustular eruption has not been described previously to our knowledge. The evidence that the present case was drug induced was the lack of a personal or family history of psoriasis and the fact that the dermatitis healed on withdrawal of the drug and recurred after it was readministered.

Departments of Dermatology,
and * Infections Diseases
University of Dijon,
C.H.U. du Bocage,
21034 Dijon Cedex, France

D.G. LAMBERT
S. DALAC
F. BEER
P. CHAVANNET*
H. PORTIER*

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Delusions of parasitosis associated with coronary bypass surgery

MADAM, The patient with delusions of parasitosis often becomes an orphan patient, dismissed by the busy physician and refusing to see a psychiatrist. We report here our experience with such a patient whom we studied for 2 years. Unimproved at the end of that time, he left us to seek care elsewhere.

A 63-year-old Caucasian man presented with a 3-year history of incessant widespread crawling and biting sensations in his skin. He blamed tiny bugs which were not directly visible on the skin, but which could be seen by him when brushed off into the wash-basin. He repeatedly demonstrated his 'bugs' by rubbing scale from his skin into a basin of water coloured blue with a food dye and illuminated by a flashlight in a darkened room. The bugs, he averred, were grossly visible and consisted of jagged legs without bodies. The patient stated that his symptoms suddenly appeared while he was in a dentist's office about 2 weeks before he underwent coronary bypass surgery, but his wife dated the onset of symptoms to the surgery itself.

He had previously consulted three dermatologists, an infectious disease specialist, a psychiatrist, an entomologist, a pathologist, a microbiologist and three parasitologists. While he was under our care, he contacted his Congressman, the National Institutes of Health, and an institute for the study of tropical diseases.

No one had been able to identify any insect, ectoparasite, fungal element, or glass wool in any of his many samples. Nonetheless, he spent most of each day cleaning, spraying and vacuuming his home to eliminate his 'pathogen'. He was also unable to sleep due to scratching.

His history revealed life-long atopic dermatitis, diabetes mellitus treated with lente-pork insulin for 13 years, two cerebro-vascular accidents 3 years earlier, and coronary bypass surgery 4 months after these. His current medications included insulin, nadolol (Corgard®), nitroglycerin (Nitro-Bid®), and nifedipine (Procardia®). Trial elimination of each of these drugs had no effect on his problem.

Physical examination revealed a generalized erythematous scaling eruption consistent with longstanding atopic dermatitis. There were no scratch marks except for minor punctuate excoriations. Routine laboratory studies were normal, including CBC, biochemistry, and urinalysis. His serum IgE was > 1000 IU/ml (normal < 80 IU/ml) and ANA was positive at a titre of 1:512. Tests for serum complement, and anti-DNA, anti-ENA, and SM antibodies were normal or negative.

Repeated examination with a 2·75 power lens of the patient's 'parasites' floating in the water revealed only corneocyte aggregates. Thirteen skin scrapings studied under light microscopy revealed no parasites. Tape strippings and two epidermal biopsies studied under scanning electron microscopy were equally unrevealing. House dust samples revealed only the housedust mite, *Dermatophagoides farinae*.

There was no apparent relation between the delusions and the state of his atopic eczema. The following systemic medications were tried without beneficial effect: acyclovir (Zovirax®), chloroquine, dapsone, cephalexin (Keflex®), ketoconazole (Nizoral®), methotrexate, metronidazole (Flagyl®), pimocide (Orap®), thiabendazole (Mintezol®), triamcinolone acetonide (Kenalog®-40) i.m. and zinc sulphate.

He obtained no relief from topical anthralin, DEET, hydrogen peroxide, nitrogen mustard, tannic acid or Vleminckx's solution.

There is a growing awareness that coronary revascularization procedures may induce major neurological syndromes.[1,2] These result from emboli (air, atheromatous debris, fat, fibrin or platelet aggregates) or cerebral hypoperfusion. Post-operative psychoses were found in 1% of 312 patients in one study,[2] with florid paranoid hallucinatory states beginning in the first 72 h post-surgery. Two of these patients still had mental aberrations at the time of hospital discharge. In another study of 3206 coronary bypass procedures, 1% of patients developed major neurological syndromes, including six with mental aberrations but no focal defects.[1]

Our patient is the first, to our knowledge, to have delusions of parasitosis associated with coronary bypass surgery. The fixed nature of his problem over 5 years suggests permanent cerebral damage. The possibility of a third stroke at the time of bypass surgery must be considered. In a survey of 282 cases of delusions of parasitosis, one was due to a stroke.[3] Indeed, the classic example of a stroke-induced delusion of parasitosis is the patient who had a 'fly with crooked wings' come to live in her ear after her first stroke.[4] She named it 'the grand capricorne,' and paralleling successive strokes, 227 little capricornes were born to spend their lives in her ear and her cheek.

The pathogenesis of delusional psychoses has now been given an anatomical basis.[5] The superimposition of a new right hemispheric infarction on a diffusely atrophied brain seems to cause delusions, which are expressed due to the left hemisphere being preserved. Therefore, our patient's two prior strokes may have set the stage for his delusion occurring in association with the cardiac surgery. In retrospect, a CT scan and NMR imaging might have been informative in our patient, and would seem indicated for patients with similar experiences.

Additional factors which may have contributed to the severity of the delusions in our patient include his diabetes mellitus[6] and generalized atopic dermatitis. Diabetes commonly favours pruritus and his dermatitis generated a steady stream of sensory impulses to the cortex, which could misrepresent them as being due to the presence of bugs. The woman with the buzzing of 227 'capricorne' flies may have been misinterpreting tinnitus.

Our patient's experience with pimocide was disappointing. His refusal to take the drug after only 1 week terminated the opportunity to use the one drug known to suppress infestational delusions.[7] Such refusal could have been a fatal decision, as this disease can and does lead to suicide.[3]

Division of Dermatology,
Department of Medicine,
Medical College of Ohio,
Toledo, Ohio 43699, U.S.A.

W.B.SHELLEY
E.DORINDA SHELLEY

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Acne conglobata in transplant patients treated with isotretinoin

MADAM, Isotretinoin has been found to have an effect on both cellular and humoral immunity, as assessed by laboratory parameters.[1] It has been shown that when skin from one mouse was transplanted to another histoincompatible mouse while the recipient was being treated with retinol and retinyl esters rejection was enhanced.[2] These findings should be borne in mind when deciding on possible treatment with isotretinoin in patients with recalcitrant acne who have received an organ graft. We report here our experience in three cases.

Case 1: A 34-year-old man with a 23-year history of juvenile diabetes and ensuing nephropathy, retinopathy, neuropathy and hypertension, underwent combined renal and pancreatic transplantation in February 1984. He was discharged on cyclosporin 7mg/kg/day and prednisolone 10 mg/day. In June 1984 he was readmitted because of an acute rejection episode of the renal graft, and was treated with intravenous pulses of methylprednisolone with a total dose of 1·25 g.

At this time previous mild acne vulgaris developed into acne conglobata affecting the face, chest and back. Treatment with tetracycline failed and isotretinoin was instituted in November 1984. There was a slow improvement and he was free from lesions in August 1985; the drug was then discontinued. During the treatment period there were no signs of rejection of either graft. The acne did not recur after isotretinoin was withdrawn.

Case 2: A 32-year-old man with a 29-year history of juvenile diabetes mellitus with supervening nephropathy and retinopathy, underwent combined renal and pancreatic transplantation in November 1984. He was discharged on cyclosporin 4·2 mg/kg/day and prednisolone 7·5 mg/day. Since adolescence he had had mild acne which worsened following immunosuppression. In June 1985, cystic and pustular acne lesions appeared on his back, chest, shoulders and face. Isotretinoin was started at 0·42 mg/kg/day and was reduced to 0·28 mg/kg/day in September 1985 because of elevated serum triglyceride levels. Serum cholesterol remained normal. Topical treatment with benzoyl peroxide gel 10% was also started. There was a slow response and isotretinoin treatment was discontinued in May 1986. During treatment there were no signs of rejection of either organ graft. The patient's acne remained well controlled after withdrawal of isotretinoin.

Case 3: A 30-year-old man with chronic glomerulonephritis received a renal transplant in February 1985. Postoperatively he had three acute rejection episodes which were treated with pulses of methylprednisolone (total dose 3 g) and rabbit-antithymocyte globulin. He was discharged on azathioprine 12·5–25 mg/day and prednisolone 30 mg/day. At the time of the acute rejection episodes, widespread acne conglobata developed. Isotretinoin treatment 0·17 mg/kg/day was started in June 1984 and continued until May 1986. His lesions cleared slowly and he became almost free of acne. During isotretinoin treatment there were no changes in triglyceride or cholesterol levels or signs of deteriorating renal function. He was subsequently maintained on topical treatment with benzoyl peroxide gel 5–10%.

Various retinoids have been shown to increase T cell numbers and augment T cell function as well as enhancing the rejection of experimental skin grafts.[1,3] Retinoid treatment in patients with organ grafts may, therefore, seem hazardous. These patients are more prone to develop severe acne conglobata because of treatment with steroids and cyclosporin.[4]

Neither during isotretinoin treatment nor during the follow-up period did we find any evidence of a detrimental effect on the grafts in our three patients. If isotretinoin or its metabolites have a detrimental effect on graft survival, we believe that this is counteracted by the immunosuppressive medication. Our patients benefitted from the treatment, which improved their quality of life. On the basis of this experience we recommend isotretinoin for the treatment of cystic acne in transplant patients. However, we suggest the treatment should be given in close collaboration with the transplant team, so that any early signs of rejection can be picked up.

Departments of Dermatology and* Transplantation Surgery,
Karolinska Institute at Huddinge Hospital,
S-141 86 Huddinge, Sweden

J.A.MARCUSSON
G.TYDÉN*

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News and Notices

The Skin Disease Research Fund

Initiation and sustenance of dermatological research is becoming increasingly difficult. It is, therefore, worth reminding colleagues that there are some alternatives to the more usual grant-giving bodies. One such is The Skin Disease Research Fund, a nationally based charitable organization devoted to fund-raising to support research on all aspects of the skin and skin diseases. The Fund does not see itself so much as an alternative to the MRC and the Wellcome Trust, but supplementary to them. The Trustees' present policy is to help investigators to get interesting projects off the ground in the hope that encouraging initial results will strengthen the investigator's ability to attract more substantial funding from major national grant-giving bodies later. Because of limited resources the Fund is reluctant to tie itself to long-term commitments especially for staff salaries, or to support travel and expenses for attendance at meetings.

Over the past 12 months the Trustees have disbursed a total of £96,000 representing eight successful applications out of a total of 18 received. Three of the larger grants are worth mentioning. Dr Colin Holden of St Helier Hospital, Carshalton, has been awarded £18,420 to extend his research, started with Dr Hanifin in Portland, Oregon, on the role of cyclic AMP phosphodiesterase as a regulatory mechanism in atopic dermatitis. Whilst recent work suggests that abnormalities of this pathway may not be specific to atopic eczema, defective function of the enzyme may explain some of the cellular and immunological abnormalities which characterize the disease.

One of the Fund's largest awards of £24,997 was made to Dr Fenella Wojnarowska of the Slade Hospital, Oxford, to study the pathogenesis of scarring in sub-epidermal bullous diseases. Knowledge of the immunopathology of these disorders is incomplete and Dr Wojnarowska proposes to use monoclonal antibodies together with immunoelectron microscopy in an attempt to elucidate mechanisms.

The Fund is particularly interested in proposals which bridge the gap between basic and applied research in the skin and has awarded Dr Irene Leigh of the London Hospital £18,000 for development of her work on the factors influencing the survival of cultured keratinocyte allografts in man. This new approach to treatment of large denuded areas of skin has been pioneered by Dr Leigh, but more work needs to be done to establish optimum conditions for growth of the grafted keratinocytes.

Much emphasis is placed by the Trustees on regular progress reports for successful applicants. To assist this process, a member of the Grants Advisory committee is normally appointed to liaise with the grant holder.

The Fund does not usually consider ad hoc applications: normally invitation to submit applications is by way of a notice in this journal.

Readers, and particularly members of the B.A.D., are asked to do what they can to attract additional donations to the Fund, by personal contact and by ensuring that the Fund's poster and leaflets are displayed in their clinic areas and waiting rooms. Donations and enquiries should be sent to the Honorary Appeal Secretary, D.K.G. Gowers, JP, ACIB, 12 Knighton Close, South Croydon, Surrey, CR2 6DP, U.K. Tel. 01-668 4300.

Skin Pharmacology Society Fifth Annual Symposium

The Fifth Annual Symposium of the Skin Pharmacology Society will be held at the Hotel Lutetia, Paris, France, on 27 and 28 May 1988. Free communications in all areas of skin pharmacology are encouraged. For further information and abstract forms please contact: Professor L. Dubertret, INSERM U 312—Department of Dermatology, Hôpital Henri Mondor 94010 Creteil, France.

Society for Cutaneous Ultrastructural Research 15th Annual Meeting

The 15th Annual Meeting of the Society for Cutaneous Ultrastructural Research will be held in Nice, France, 20–21 May, 1988. For further information please contact: Jean-Paul Ortonne M.D., Hôpital Pasteur, B.P. 69, 06002 Nice Cedex, France, Telephone:93.81.95.16

Third National Symposium for Dermatology Clinical Assistants

The third National Symposium for Dermatology Clinical Assistants will be held at the Grand Hotel, Eastbourne, U.K. from 15–17 April 1988. Kirby-Warrick Pharmaceuticals have kindly agreed, once more, to sponsor the event. Please note that Section 63 approval has been given for this symposium. For further details please contact: Dr R.A.C. Graham-Brown, Consultant Dermatologist, Department of Dermatology, Leicester Royal Infirmary, Infirmary Close, Leicester LE1 5WW, U.K.

Dermatopathology Self-Assessment Workshop

A Dermatopathology Self Assessment Workshop will be held at the Cleveland Clinic Foundation, Cleveland Foundation, Cleveland, Ohio, U.S.A. on 29 October 1988. For further information contact the course director, Dr Wilma F. Bergfeld, Cleveland Clinic Foundation, 9500 Euclid Avenue, Cleveland, Ohio 44106 U.S.A. Telephone (216) 444-5722.

Dermato-Pathological Self Assessment Course

A Dermato-Pathological Self Assessment course will be held at Guy's Hospital, London on Friday 22 and Saturday 23 April 1988. A wide range of material will be studied and the programme will be of interest to registrars and consultants in dermatology and pathology. For further information contact: Dr M. L. Price, Department of Dermatology, Guy's Hospital, London SE1 9RT. Tel. 01 407 7600 ext. 2539.

Erratum

Sun Chee-Ching, Lu Yau-chin, Fung Lee Evelyn, Nakagawa Hidemi. Naevus fusco-caeruleus zygomaticus. 1987; **117**; 545–53. Table 2 (Number and distribution of pigmented spots in 87 cases of naevus zygomaticus) should read as follows:

No. of spots		<5	6–10	11–15	16–20	21–25	26–30	>31	Mean ± SD
No. of cases	Right	7	35	17	6	10	9	3	15·1 ± 8·6
	Left	5	36	17	10	9	5	5	15·2 ± 8·9

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High endothelial venules in involved and uninvolved psoriatic skin: recognition by homing receptors on cytotoxic T lymphocytes

MADALENE C.Y.HENG, SUNI G.ALLEN AND D.G.CHASE

Division of Dermatology, Department of Medicine, UCLA San Fernando Valley Internal Medicine Program, Veterans Administration Medical Center, Sepulveda, California, U.S.A.

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SUMMARY

We report the presence of tannic acid staining material in the intercellular spaces between adjacent endothelial cells of the high endothelial venules (HEVs) in psoriatic skin, which appears to be recognized by T8 (cytotoxic/suppressor) lymphocytes, as the presence of HEVs was found to be related to the presence of T8 lymphocytes in the epidermis. As HEVs with similar tannic acid staining material were also observed in peripheral lymph nodes, we suggest that this material may serve as a marker for HEVs recognized by the T8 lymphocyte subset. T8+ lymphocytes were found in the epidermis at 5 min and 1 h after tape-stripping of uninvolved skin of psoriatic patients with active disease, but not until 24 h in a psoriatic patient in remission. The prior existence of HEVs in uninvolved psoriatic skin could account for the rapid egress of T8 lymphocytes from the vasculature to the epidermis in response to trauma.

Blood vessel abnormalities have been considered to be an integral part of psoriasis.[1] These vascular changes precede the development of overt histological psoriasis.[1–3] Occlusion of the vascular bed for 24 h after experimental injury to the skin appears to prevent psoriasis from developing in uninvolved psoriatic skin.[4] Ultrastructurally, the proliferative vascular component in psoriatic skin is venous rather than arterial, with proliferation of the post-capillary venular component replacing much of the capillary loop.[5,6] Conversely, it has been noted that a reversion to an arteriolar configuration preceded a decrease in epidermal labelling index in psoriatic skin treated by the Goeckerman regimen.[5]

Multiple fenestrations have been noted in both the basal lamina and in the walls of the post-capillary venules.[1] Recently, the size of these fenestrations was studied by computer analysis, and they were found to be large defects, 4–5 μm in diameter.[7] We believe that these gaps may represent defects created by the egress of lymphocytes from the vessels into the tissues, as mononuclear cells have been observed frequently within these defects.[6]

Correspondence: Madalene C.Y.Heng, M.D., UCLA School of Medicine, Division of Dermatology, Veterans Administration Medical Center, 16111, Plummer, Sepulveda, CA 91343, U.S.A.

It has become increasingly apparent that the migration of lymphocytes from the blood into the lymph nodes occurs via a system of specialized microvasculature, the high endothelial venules (HEV), a structure easily recognizable on electron microscopy.[8,9] HEVs are normally situated only at the level of post-capillary venules in lymph nodal tissue (peripheral lymph nodes and Peyers patches), and are the means whereby lymphocyte recirculation from the blood to the lymph nodes is possible, thus facilitating the immune process by allowing lymphocytes to interact with antigen, with other lymphocyte subsets, and to remove tumour cells and cells affected by viruses. These activated lymphocytes eventually return to the blood stream via the lymphatic system.[10] It has been observed that lymphocytes adhere and bind to these specialized endothelia.[11] Binding of the lymphocytes to HEVs precedes migration of these cells through these vessels, and is thought to involve specific receptors on the lymphocytes which recognize equally specific antigens present on the endothelial cells which form the HEV.[12]

We report here a study of a specialized variety of HEV present in involved psoriatic skin but occasionally also found in uninvolved psoriatic skin.

METHODS

Biopsies

Biopsies were taken from patients attending the dermatology clinics at the Veterans Administration Medical Center. In 12 psoriatic patients biopsies were taken from active, untreated lesions and from uninvolved skin (10 cm or more from the nearest psoriatic lesion). Biopsies of uninvolved skin were taken from two psoriatic patients in remission.

Control biopsies of involved and uninvolved skin were taken from four patients with a beta-blocker induced psoriasiform eruption, four patients with eczema, three with Darier's disease, and two patients with other psoriasiform drug eruptions (quinine, one patient; Bactrim, one patient).

Light and electron microscopy

Four mm punch biopsies were taken and divided longitudinally. One half was fixed in 10% neutral buffered formalin and processed for light microscopy. Paraffin sections were stained with haematoxylin and eosin. The other half was fixed in 2·5% glutaraldehyde, buffered to pH 7·3 with 0·1 M sodium cacodylate, post-fixed in osmium tetroxide, treated en bloc with tannic acid, dehydrated in ethanol and propylene oxide, and embedded in a mixture of Epon 812 and Araldite 502. Silver sections (70–80 nm thick) were cut on a Sorvall MT2B ultramicrotome with a diamond knife (Dupont) and examined with a Philips EM 201 transmission electron microscope. Three to six grids were cut from each specimen and 30–60 photographs taken per grid. From each biopsy 1500 to 2000 μm of epidermal length, and the equivalent length of dermal tissue was examined and the results expressed per 500 μm epidermal length.

Immunocytochemistry

Adjacent 4 mm punch biopsies were taken for immunocytochemistry. These were embedded in OCT compound (Tissue-Tek), snap-frozen in liquid nitrogen and stored at −70°C. Serial cryostat sections (4 μm) were mounted on gelatin-coated slides and air-dried for 30 min at room temperature. The mounted sections were then freeze-dried for 4 h, fixed in acetone for 20 min at room temperature, and air-dried for 5 min prior to immunostaining. Monoclonal antibodies, as listed in Table 1, were added to the sections, which were then incubated for 60 min at room temperature, washed in phosphate buffered saline (PBS) pH 7·6, and overlaid with peroxidase-

TABLE 1. Monoclonal antibodies used in the study

Antibody	Dilution	Specificity	Supplier
Leu 2a	undiluted	cytotoxic/suppressor T cells	Becton-Dickinson
Dako-T8	undiluted	cytotoxic/suppressor T cells	Dako
Leu 3a	undiluted	T helper cells	Becton-Dickinson
Dako-T4	undiluted	T helper cells	Dako
Leu 14	1:20	B lymphocyte surface antigens	Becton-Dickinson
Dako-macrophage	1:10	Macrophages	Dako

conjugate. The sections were washed with PBS and the peroxidase colour reaction developed using diaminobenzidine. Negative controls from all patients were processed as above, omitting the monoclonal antibodies. Further controls were stained with kappa chain monoclonal antibodies and cytokeratin antibodies to monitor the procedure.

Tape-stripping studies

Tape stripping was carried out on the uninvolved back skin of three psoriatic patients (two with active lesions, 20–30% skin involvement, and one in remission) and three control patients (two with naevi and one with seborrhoeic keratoses) as described previously.[13] Forty tape strippings were performed in each subject and erythema was obtained in all cases at the end of the procedure. Biopsies were taken 5 min, 1 h and 24 h after tape stripping, and processed for light microscopy, electron microscopy and immunocytochemistry.

Lymph node studies

Clinically normal lymph nodes from surgical specimens were processed for electron microscopy, using tannic acid, and the vascular morphology studied under a transmission electron microscope.

RESULTS

The results are summarized in Table 2.

Electron Microscopy

In all biopsies from involved psoriatic skin, the post-capillary venules, which in non-psoriatic patients were characterized by a single layer of somewhat flattened endothelial cells (Fig. 1), and easily recognized by the presence of multilayered basal lamina, had been replaced by specialized endothelial cells of the high endothelial venule type (Fig. 2a). In addition, the psoriatic HEVs were characterized by the presence of tannic acid-staining material (TASM), mainly localized to the intercellular spaces between the tall endothelial cells (Fig. 2a). This material appeared to be secreted by vesicular structures lining the intercellular spaces between these specialized endothelial cells (Fig. 2a), as the TASM in these vesicles was often continuous with the TASM present in the intercellular spaces (Fig. 2b). Psoriatic HEV resembled HEV from peripheral lymph nodes with respect to the presence of tall endothelial cells and of TASM in the intercellular spaces between these cells. However, they differed in that the TASM in psoriatic HEVs often filled the entire space between the adjacent endothelial cells (Fig. 2b), whereas in lymph node HEVs, the intercellular spaces between the adjacent endothelial cells were never

TABLE 2. Summary of the results obtained in the study

	Psoriatic patients	Controls
Active, untreated disease	$n=12$	$n=13$
Involved skin		
T8+ lymphocytes in epidermis	All cases	Not detected
Capillaries (no./500 μm epidermal length) (mean ± SE)	1·6 ± 0·1 range: 1–2	2·7 ± 0·7 range: 2–4
PCV (no./500 μm epidermal length) (mean ± SE)	0	5·7 ± 0·9 range: 4–7
HEV (no./500 μm epidermal length) (mean ± SE)	11·3 ± 0·7 range: 8–16	0
TASM containing vesicles	8 of 12 biopsies (67%)	0 of 13 biopsies
Uninvolved skin		
T8+ lymphocytes in epidermis	0	0
Capillaries (no./500 μm epidermal length) (mean ± SE)	2·5 ± 0·2 range: 1–3	3·7 ± 0·7 range: 3–5
PCV (no./500 μm epidermal length) (mean ± SE)	1·0 ± 0·2 range: 1–3	2·4 ± 0·1 range: 2–3
HEV (no./500 μm epidermal length) (mean ± SE)	3·2 ± 0·5 range: 2–4	0
TASM containing vesicles	4 of 12 biopsies (33%)	0 of 13 biopsies
Disease in remission (Uninvolved skin)	$n=2$	
T8+ lymphocytes in epidermis	Not detected	
Capillaries (no./500 μm epidermal length) (mean and range)	2·5 (1–2)	
PCV (no./500 μm epidermal length) (mean and range)	4·5 (4–5)	
HEV (no./500 μm epidermal length) (mean and range)	1·5 (1–2)	
TASM containing vesicles	Not detected	

PCV = post capillary venules; HEV = high endothelial venules; TASM = tannic acid staining material.

observed to be completely filled with TASM (Figs 3a and b). In addition, the microvesicular structures containing the TASM were not as darkly stained in lymph node HEVs (Fig. 3b).

Uninvolved psoriatic skin revealed the presence of HEVs in all biopsies, with TASM present in some, but not all of the intercellular spaces between adjacent endothelial cells (Fig. 4). HEVs with vesicles containing TASM were observed less frequently in uninvolved psoriatic skin than in lesional psoriatic skin (4 of 12 and 8 of 12 biopsies, respectively).

HEV type post-capillary venules greatly outnumbered the capillaries in involved psoriatic skin (Table 2). In addition, in many biopsies from involved psoriatic skin, the HEVs were observed to directly underlie the epidermal basement membrane (Fig. 5). This was also observed in uninvolved psoriatic skin in three patients.

Immunocytochemistry

Using monoclonal antibodies to detect lymphocyte subsets in biopsies of lesional skin from

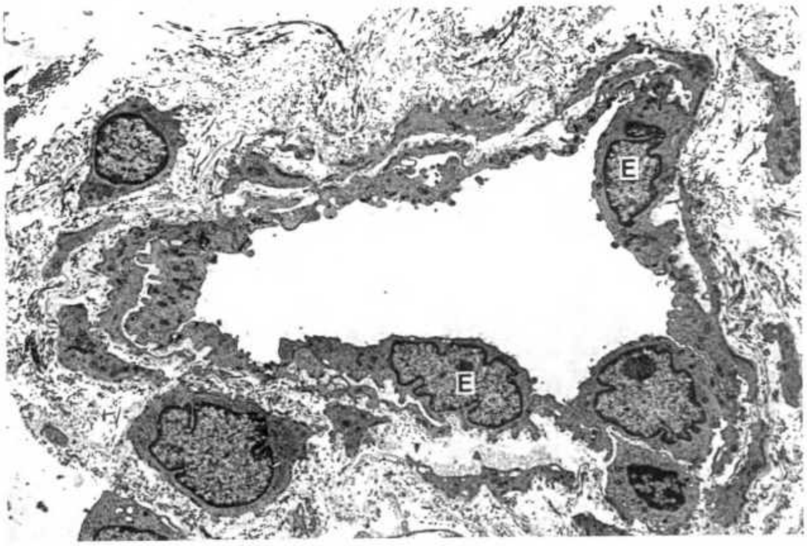

FIGURE 1. Post-capillary venule in lesional skin from a patient with a psoriasiform eruption associated with atenolol, showing the presence of somewhat flattened endothelial cells (E) (Original × 7,500).

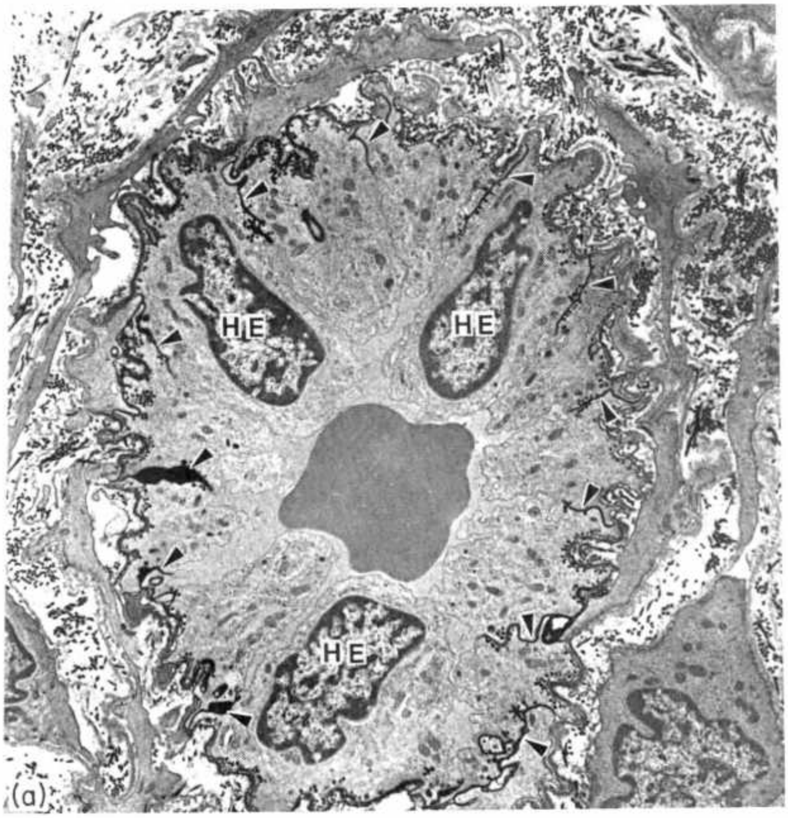

FIGURE 2. (a) Biopsy from an active psoriatic lesion, showing a high endothelial venule, lined by a single layer of tall endothelial cells (HE). Note the presence of tannic acid staining material (arrows) filling the intercellular spaces between adjacent endothelial cells (Original × 7,500).

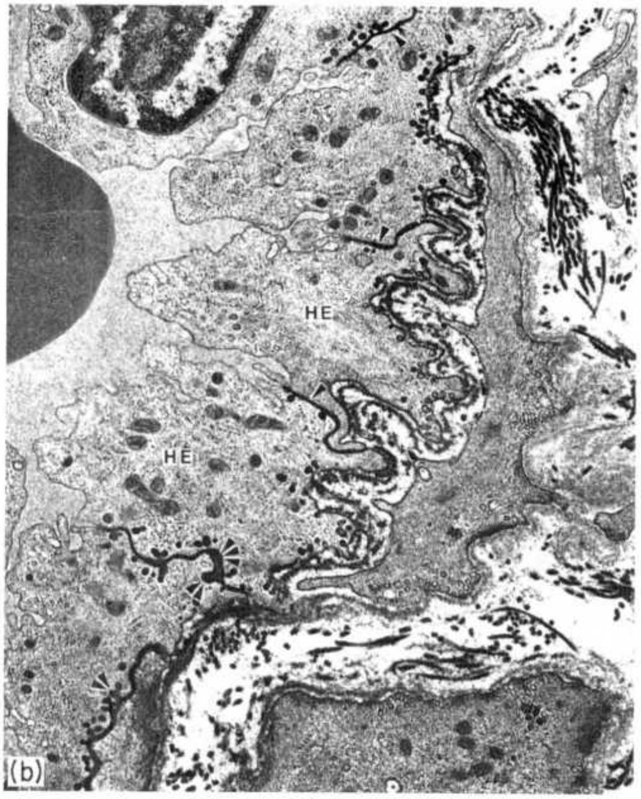

FIGURE 2. (b) Higher magnification of a high endothelial venule from an active psoriatic lesion showing tannic acid staining material (single arrows) mainly located in the intercellular spaces between adjacent tall endothelial cells (HE), completely filling these spaces, even where there are dilatations (triple arrows). Note the presence of vesicles (double arrows) filled with tannic acid staining material that appears to be continuous with that in the intercellular spaces. (Original × 17,500).

psoriatic patients and controls, we found T8 and Leu 2a positive (cytotoxic/suppressor T cells) in the epidermis in psoriatic skin (Fig. 6), but not in the control. T4 and Leu 3a positive cells (T helper cells) were mainly found in the dermis, but also in the epidermis, in both psoriatic and control skin. Macrophages were present around the blood vessels and in the upper dermal infiltrate in involved psoriatic and control skin, while B lymphocytes were not detected.

Tape-stripping studies

T8+ lymphocytes were found in the epidermis and dermis of uninvolved skin 5 min after the start of tape-stripping in one patient with active psoriasis (Fig. 7a), 1 h after tape-stripping in the second patient with active psoriasis, and 24 h after tape-stripping in the psoriatic patient in remission (Fig. 7b). T8+ lymphocytes were not found in the epidermis or dermis in any of the control biopsies.

T4+ cells were also found in the epidermis and in a perivascular distribution in the dermis in the same biopsies from the psoriatic patients. In addition, they were found in a perivascular distribution in the 24 h biopsy from one of the controls.

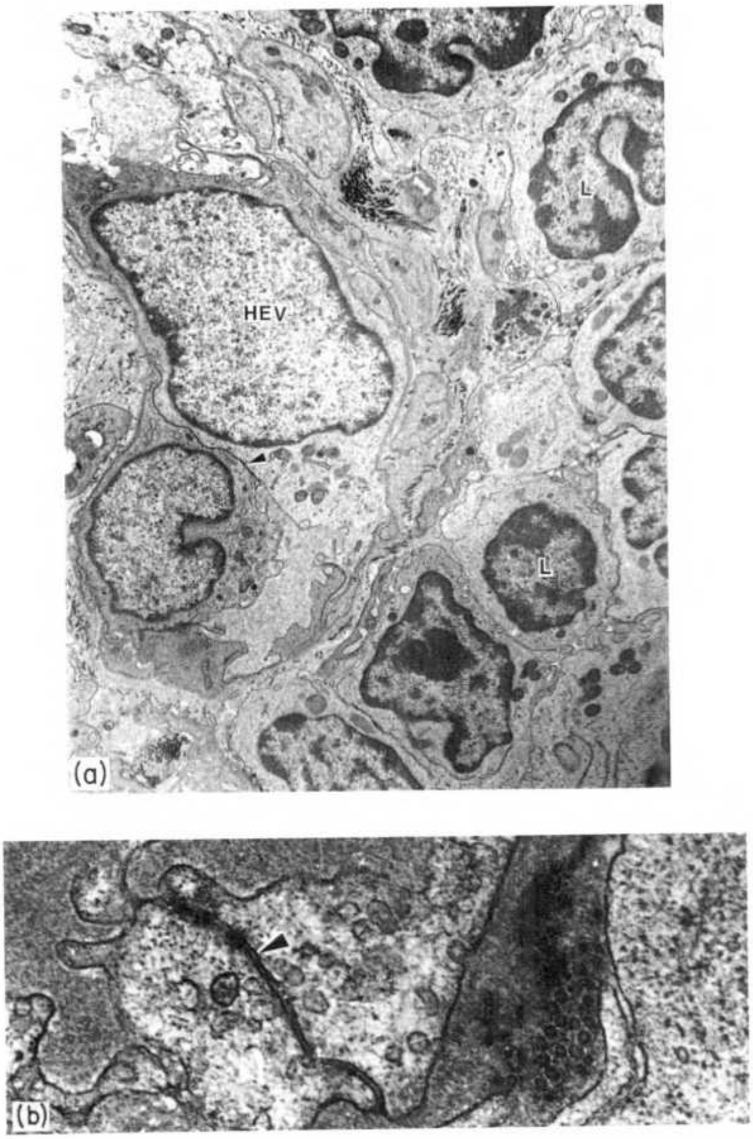

FIGURE 3. (a) Section of a peripheral lymph node, showing a high endothelial venule (HEV) with tannic acid staining material (single arrow) in the intercellular space between adjacent endothelial cells. L = lymphocytes. (Original × 11,500). (b) Higher magnification, of (a). The tannic acid staining material (single arrow) does not completely fill the intercellular space between adjacent endothelial cells. (Original × 17,500).

DISCUSSION

Recirculating lymphocytes leave the blood stream for lymph nodal tissue via a system of specialized venules, the high endothelial venules, distinguished by endothelium composed of tall cells.[8,9] This selectivity has been attributed to the presence of surface receptors on lymphocytes which recognize specific antigens on the endothelial cells of these specialized

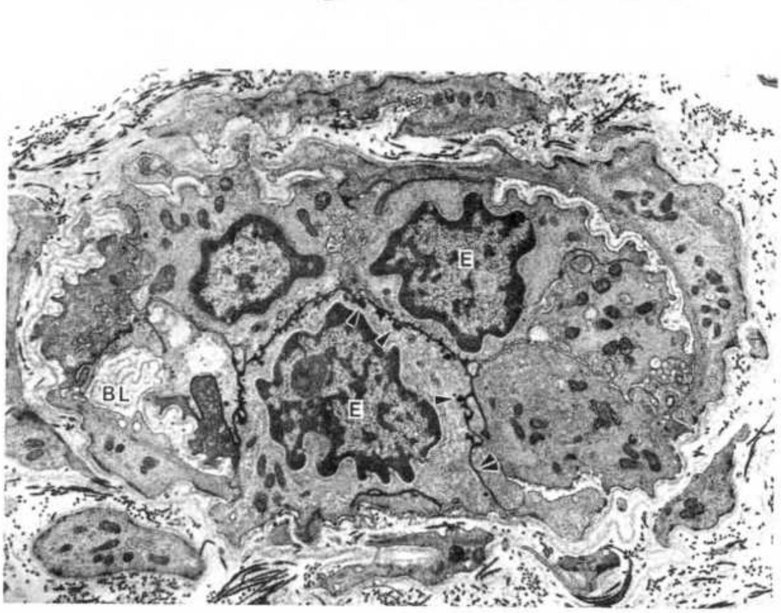

FIGURE 4. Biopsy of uninvolved skin from a psoriatic patient with active disease, showing a high endothelial venule lined by tall endothelial cells (E). Note the presence of tannic acid staining material (double arrows) in some but not all the intercellular spaces between adjacent endothelial cells, and the presence of vesicles lining the intercellular spaces (single arrows), filled with the same material. The apparent involvement of the apical portions of the endothelial cells is due to the section being oblique. BL = basal lamina. (Original × 11,500).

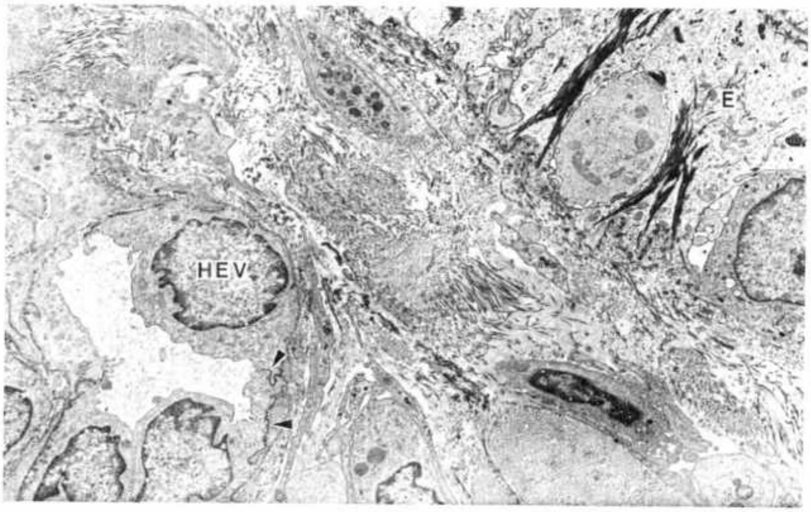

FIGURE 5. Biopsy from an active psoriatic lesion, with a high endothelial venule (HEV) situated just below the epidermis (E). Note the presence of electron dense material in the intercellular spaces between adjacent endothelial cells, and the presence of vesicles lining these spaces, filled with the same tannic acid staining material (single arrows). (Original × 5,000).

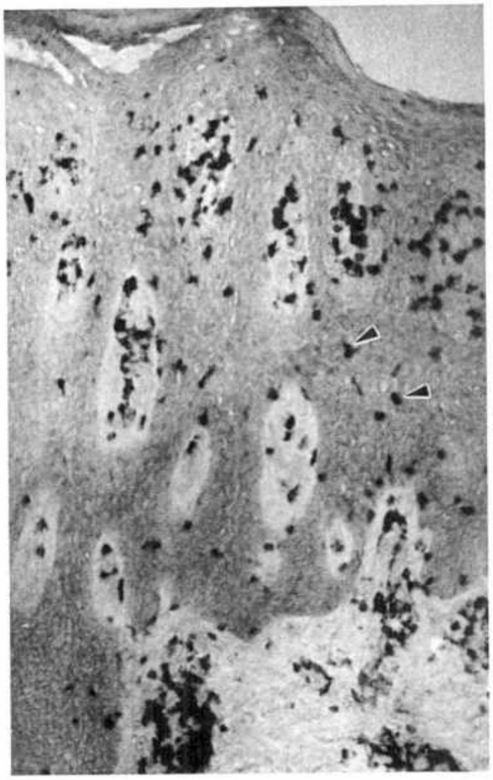

FIGURE 6. Immunocytochemical staining of an active psoriatic lesion, showing the presence of Leu 2a + lymphocytes in the epidermis (arrows). (Original × 200).

venules.[14,15] Evidence for the existence of such a cell-surface recognition system has been provided by the development of a rat monoclonal antibody against murine lymphocytes (MEL-14), which detects a cell-surface determinant present only on those cells which bind to peripheral lymph node HEVs.[16] MEL-14 has been observed to block *in vitro* binding and *in vitro* homing of lymphocytes to peripheral lymph node HEVs, but not Peyer's patches HEVs, thus suggesting that several lymphocyte homing receptor-HEV antigen systems exist to direct certain lymphocytes to specific areas.

In the present study we found HEVs of the psoriatic type, with increased amounts of tannic acid staining material in the intercellular spaces between endothelial cells, in all active psoriatic lesions examined, and in some biopsies of uninvolved skin from patients with active psoriasis. The presence of HEVs in uninvolved psoriatic skin suggests that the presence of these specialized venules is not secondary to the skin eruption. The greater numbers of HEVs found in active psoriatic lesions suggest that formation of HEVs can be induced by external stimuli, and this could account for the increased reactivity of uninvolved skin of psoriatic patients with active disease, compared with those in remission.

Tape-stripping of uninvolved psoriatic skin revealed the presence of T8 positive (cytotoxic/suppressor) lymphocytes in the epidermis 5 min after tape-stripping in one patient. HEVs were also found in the skin of this patient. These findings suggest that homing receptors on the T8

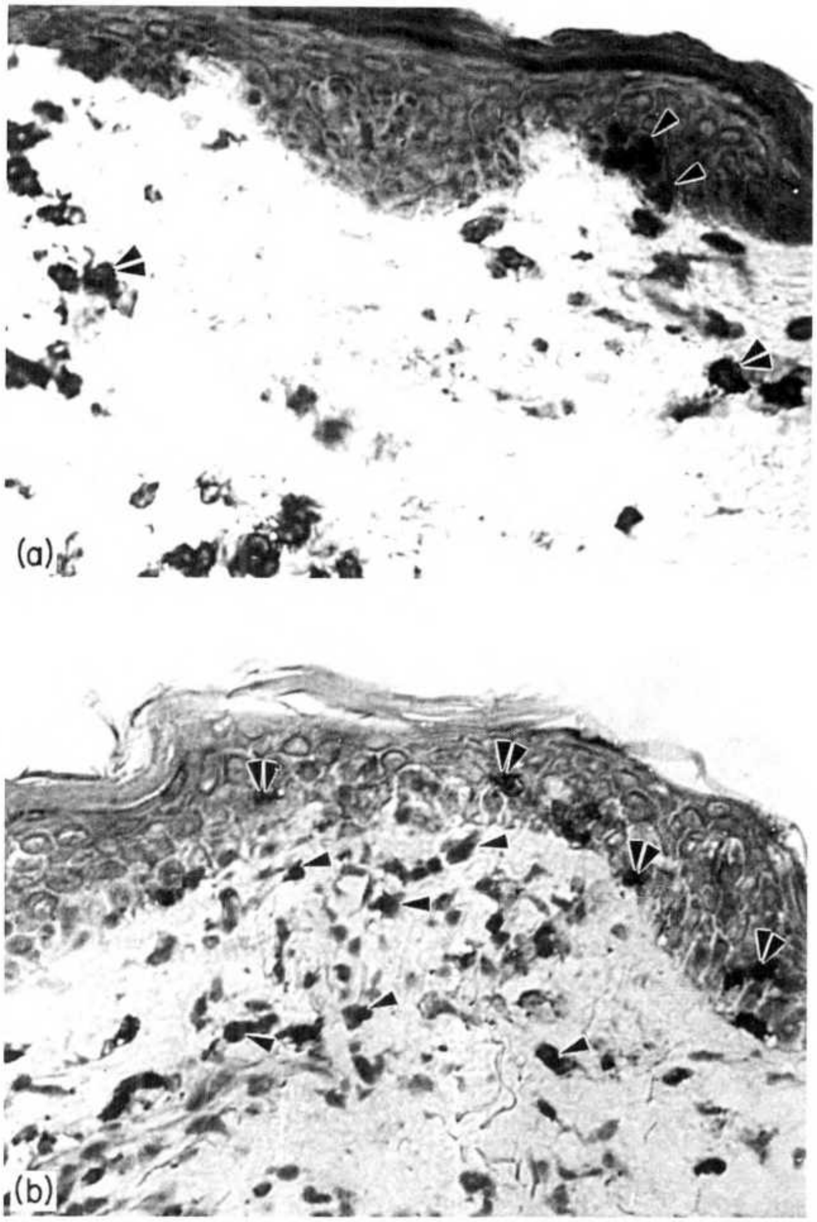

FIGURE 7. (a) Immunocytochemical staining of uninvolved psoriatic skin 5 mins after tape-stripping, showing the presence of Leu 2a + lymphocytes in the dermis (double arrows) and in the epidermis (single arrows). (Original × 600). (b) Immunocytochemical staining of uninvolved skin from a psoriatic patient in remission 24 h after tape-stripping. Leu 2a + lymphocytes are present in the epidermis (double arrows) and in the dermis (single arrows). (Original × 550).

lymphocytes recognize a specific antigen present on the endothelia of psoriatic HEVs, and also that HEVs with this antigen must be present in uninvolved skin at the time of tape-stripping, since it takes many hours for the rough endoplasmic reticulum to synthesize protein molecules. The presence of TASM in the intercellular spaces between the endothelial cells of the psoriatic HEVs may reflect the presence of the antigen recognized by the T8 lymphocyte subset, and may well serve as a marker for this vascular antigen.

The presence of apparently similar TASM in the intercellular spaces between adjacent endothelial cells of peripheral lymph node HEVs and psoriatic HEVs, suggests similar, or identical, HEV antigen-lymphocyte homing receptor systems in the two. If the TASM represents the HEV antigen, the presence of greater amounts of TASM in psoriatic HEVs, perhaps due to increased synthesis by the TASM containing vesicles, may allow greater numbers of T lymphocytes to leave the blood vessels for the epidermis by presenting more binding sites.

The differentiation of papillary capillaries, which are normally situated beneath the basement membrane, into psoriatic HEVs in both involved and uninvolved skin in psoriatic individuals may account for the rapid migration of cytotoxic T8 lymphocytes into the epidermis after tape-stripping of uninvolved psoriatic skin. The migration of T8 lymphocytes into the epidermis 5 min to 24 h after tape-stripping is essential for the induction of psoriasis by the Koebner phenomenon, as previous studies have shown that vascular occlusion of the capillary bed for 24 h after experimental injury prevented psoriasis from developing in uninvolved psoriatic skin.[3] Our finding of a shorter lag period between tape-stripping and the appearance of T8 lymphocytes in the epidermis in uninvolved skin of psoriatic patients with active lesions compared with that in uninvolved skin of a psoriatic patient in remission may be due to the more numerous HEVs present in uninvolved skin of patients with active psoriasis. These results are consistent with our previous findings, in that the earliest changes which could be seen on electron microscopy occurred 2 min after tape-stripping.[13]

The antigenic determinant recognized by the monoclonal antibody MEL-14, which is only present on lymphocytes which bind to peripheral lymph node HEVs[16] has been reported to reside in the carboxyl terminal 13-amino acid proteolytic peptide of ubiquitin.[12] It would thus appear that ubiquitin, which is currently better known for its role in protein degradation[17] has an additional function in lymphocyte binding and degradation. It has further been reported that MEL-14 may recognize the subset of cortical thymocytes which are resistant to cortisone.[18] As both T3 and T4 lymphocyte subsets are highly sensitive to corticosteroids, it appears that MEL-14 may recognize the T8 (cytotoxic/suppressor) lymphocyte subset. In other words, the homing receptor on the lymphocytes of the cytotoxic/suppressor subset, recognized by MEL-14, has been shown to recognize lymph node HEVs,[16] which in turn are identified by the presence of TASM in the intercellular spaces between the endothelial cells, as well as by the presence of tall endothelium lining the post-capillary venules. The presence of the TASM may thus be a marker for HEVs of the lymph node variety, recognized by cytotoxic suppressor lymphocytes. These concepts are consistent with our observations, both from tape-stripping studies of uninvolved psoriatic skin and from immunocytochemical and electron microscopic studies of active, untreated psoriasis, that the presence of T8 lymphocytes in the epidermis was associated with the presence of specialized HEVs that were characterized by the presence of electron dense material between the endothelial cells. This material appeared to be more abundant in psoriatic HEVs than in peripheral lymph node HEVs, and may be secreted by vesicles lining the intercellular spaces between adjacent endothelial cells. The prior presence of HEV in uninvolved psoriatic skin would account for the rapid egress of the cytotoxic/suppressor

T lymphocytes from the blood vessels into the epidermis after tape-stripping of psoriatic skin, which was not found in control skin where no HEVs were detected.

It is currently recognized that cytotoxic/suppressor T lymphocytes are formed as a normal part of the mammalian response to foreign cell-surface antigens. Among these antigens are class I histocompatibility antigens, viruses and tumour-associated antigens. Although this system involves lysis of allogeneic cells,[19] it is believed to be the means whereby cytotoxic cells destroy virally infected autologous tissue or syngeneic tumour cells, as it has been shown that viruses and other antigens (including chemicals and synthetic haptens) elicit the production of cytotoxic/suppressor lymphocytes which lyse only those virus-infected target cells that display class I histocompatibility antigens identical to those on the cytotoxic cells.[20] The ability of trauma to induce the infiltration of cytotoxic/suppressor lymphocytes into the epidermis, suggests that this may be the mechanism of removal from the epidermis of cells that are damaged, not only by viruses and chemicals, but also by physical trauma.

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The laminate capillary and its wall of multiple basement membranes

W.B.SHELLEY, E.DORINDA SHELLEY AND
VERONIKA BURMEISTER*

Division of Dermatology, Department of Medicine, Medical College of Ohio, Toledo, Ohio 43699, U.S.A. and
Department of Dermatology,* University of Illinois, College of Medicine, Peoria, Illinois 61656, U.S.A.

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SUMMARY

Cutaneous telangiectatic vessels from two cases of unilateral dermatomal superficial telangiectasia were shown under electron microscopy to be venular capillaries with thick walls composed of multiple basement membranes. We suggest that this special capillary structure, seen in a variety of telangiectatic and disease states, should be called the 'laminate capillary'.

Capillaries exhibiting an outer wall of multiple basement membranes (basal laminae) are unusual and unnamed, but have been reported in many conditions, including diabetes, porphyria, and many types of telangiectasia and angiomas.[1–13] The walls of these distinctive vessels can be seen clearly on transmission electron microscopy, but on light microscopy appear only as dim hyaline sheaths.

We present examples from two patients with unilateral dermatomal superficial telangiectasia, and propose the name 'laminate capillary' for this unique vessel.

CASE REPORTS

Case 1

A 22-year-old man presented with blotchy red patches on the lateral right upper arm which had been present for 8 years. During the last year, the lesion had slowly enlarged. He denied any other cutaneous or medical problems. No other family members had similar skin changes. Examination revealed a faint irregular erythematous macule, measuring 10 cm on its longest axis, with similar smaller islands extending medially in a neural distribution towards the axilla. The lesions resembled a naevus flammeus and a diagnosis of unilateral naevoid telangiectasia (unilateral dermatomal superficial telangiectasia) was made. A full blood count, biochemistry, ASO titre, ANA, and cold agglutinins were normal.

A skin biopsy revealed telangiectasia consisting of scattered small vessels with slight

Correspondence: Dr W.B.Shelley.

thickening of the walls. There were no other abnormalities. Immunofluorescence was negative for IgG, IgA, IgM, C_3, C_4, C_{1q}, fibrinogen and albumin.

Scanning electron microscopy of critical point dried specimens (gold coated, and examined under a JEOL-35 unit, with 45° tilt) showed dilated blood vessels with normal endothelial walls. Transmission electron microscopy after glutaraldehyde fixation, dehydration, uranyl acetate-

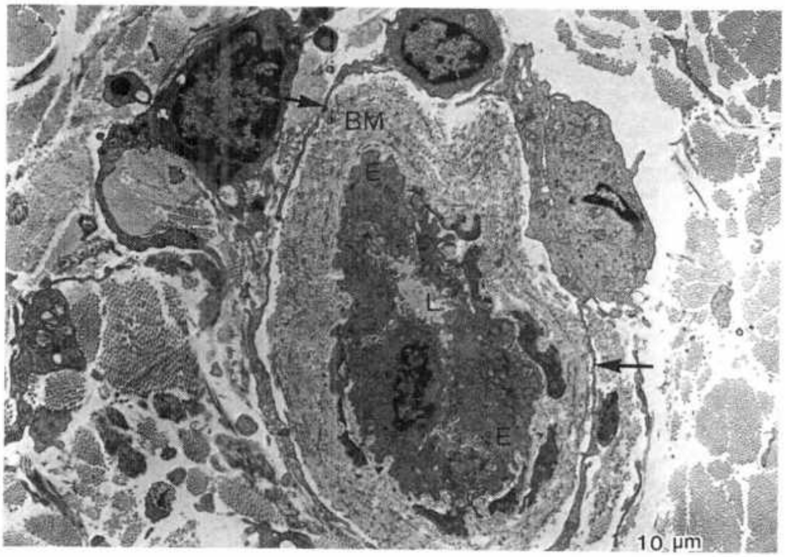

FIGURE 1. Transmission electron micrograph of a laminate capillary from Case 2, showing a small lumen (L), endothelial cell (E) and a thick wall of reduplicated basement membranes (BM). Veil cells (arrows) surround the vessel.

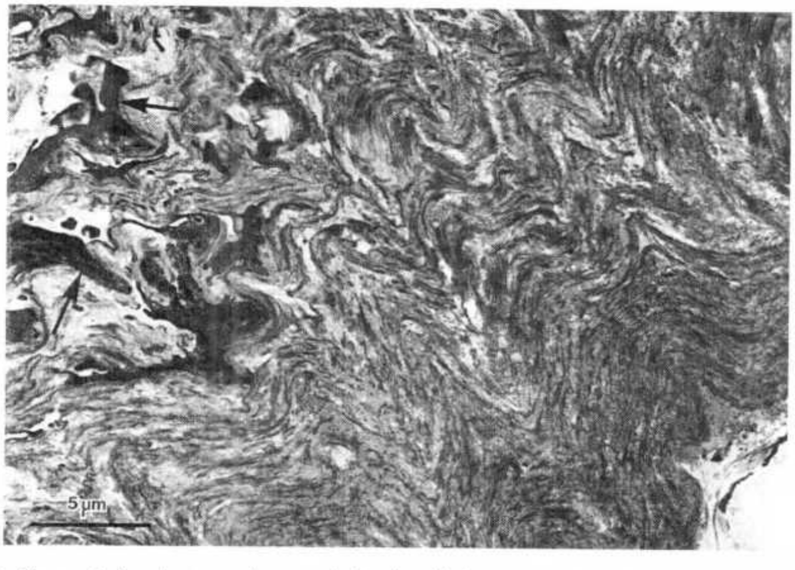

FIGURE 2. Transmission electron micrograph showing higher power view of the multilayered wall of a laminate capillary from Case 1, showing at least 20 concentric bands of basement membrane. Pericytes can be seen near the lumen (arrows).

lead citrate staining. Epon embedding and examination under a JEM 100 C unit (JEOL USA, Inc., Peabody, MA) showed numerous dilated capillaries lined with endothelium surrounded by wide concentric bands of reduplicated basement membranes (see Figs 1 and 2).

Case 2

A 25-year-old pregnant woman presented with blotchy red patches on the left shoulder, the extensor surface of the left arm, and the dorsum of the left hand. Telangiectatic vessels were prominent. She had first developed the telangiectasia 4 years previously during her first pregnancy, with subsequent fading of the lesion after delivery. At presentation, during her second pregnancy, the lesion was markedly more extensive. No other family members had similar lesions. A diagnosis was made of unilateral dermatomal superficial telangiectasia sensitive to the elevated oestrogen levels of pregnancy.

A full blood count, biochemistry, and ANA were normal. Skin biopsy revealed only some dilated capillaries. Transmission electron microscopy showed capillaries with numerous reduplicated basement membranes (Figs 1 and 2).

DISCUSSION

The capillary with multiple basement membranes (basal laminae) appears to us to be a structure in search of a name. It is a unique anatomical structure identifiable on electron microscopy and possesses unique functional and physiological properties. It is not a conventional capillary with a thin single basement membrane sheath. It is not an arteriole with a wall of smooth muscle, nor a venule with a wall of pericytes and veil cells.[14,15] Accordingly, we propose that it be named the 'laminate capillary.'

Capillaries with multiple basement membranes appear in a variety of pathological states. To our knowledge they have been recorded only once before in unilateral superficial telangiectasia,[4] but have been observed in many other types of telangiectasia:[1–3,5,6,13] hereditary haemorrhagic telangiectasia (Osler-Weber-Rendu), progressive ascending telangiectasia, nasal telangiomas of habitual nose bleeders, senile or cherry angiomas (De Morgan's spots), haemangiomas of the POEMS syndrome, naevus flammeus, and mat telangiectasia with or without scleroderma. Actinically damaged skin, in normal individuals and porphyria patients, also regularly contains such vessels.[7,8] While most commonly reported in diabetes mellitus, they are also seen in leprosy, polymyositis and eosinophilic fasciitis.[9–12]

Basement membranes are sheets of extracellular matrix secreted by parenchymal cells which interface with connective tissue,[16] including epithelial and mesothelial cells, as well as endocrine, muscle, fat, and Schwann cells. The source of the replicated basement membrane of the laminate capillary is presumed to be the endothelial cell. In culture, endothelial cells regularly synthesize type IV collagen and glycoproteins, the constituents of the basement membrane.[17] Furthermore, in experimental protoporphyria in mice, irradiation of the skin destroys the endothelial cells, which are then replaced by new endothelial cells that synthesize their own basement membrane.[18] Weekly bursts of radiation over a 28-week period induced formation of a tube consisting of 10 concentric basement membranes encircling the capillary. Such multilaminate basement membranes have been viewed as rings, each resulting from a new endothelial cell population.[19,20] This single endothelial cell–single basal lamina concept suggests that the regeneration of endothelial cells induces the replication of basement membrane.

Presumably, collagenase and other degradation systems exist for removal of non-functioning redundant basement membranes. We have reported disappearance of the multi-laminate basement membrane in one case of progressive ascending telangiectasia during ketoconazole therapy.[2] Similarly, in diabetes the replicate basement membranes disappear when good metabolic control is achieved.[21]

The basement membrane in capillaries provides structural support for a thin endothelial membrane which otherwise might rupture.[22] The basement membrane also serves as scaffolding for reconstruction of the endothelial capillary tube after injury, and provides a substrate for orderly positioning of new cells in a tubular pattern.[23] In addition, the basement membrane plays a role in fluid and metabolic exchange, which is impeded by the presence of the thicker laminate wall.[24] Further studies should confirm the view that the transfer of cells, fluids, and antigens is altered significantly in these vessels. Immune responses directed towards the basement membrane might single out the 'laminate capillary' as a special target.

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Modulation of phospholipase A_2 activity in extracts of lesion-free psoriatic epidermis by alkaline phosphatase and a protein phosphatase inhibitor

P.H.CARTWRIGHT*, A.ILCHYSHYN*, ELIZABETH ILDERTON* AND H.J.YARDLEY†

* Department of Dermatology, North Staffordshire Hospital Centre, Stoke-on-Trent and † Department of Biological Sciences, University of Keele, Staffordshire, U.K.

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SUMMARY

Phospholipase A_2 activity is raised in non-lesional psoriatic epidermis compared with normal epidermis. It has been shown that the activity of this enzyme is controlled by an inhibitory protein the inhibitory effect of which is increased by dephosphorylation. Treatment of epidermal extracts with alkaline phosphatase reduced the phospholipase A_2 activity, both in normal and in lesion-free psoriatic epidermis. Inclusion of pyrophosphate, a protein phosphatase inhibitor, in the homogenizing medium caused the activity of phospholipase A_2 in epidermal extracts from normal and lesion-free epidermis to be raised to the same high level. These results are consistent with the hypothesis that the raised phospholipase A_2 activity in psoriatic epidermis is due to hyperphosphorylation of an endogenous inhibitor as a result of defective control of a phosphorylation/dephosphorylation mechanism. The relevance of these findings to other work is discussed.

Phospholipase A_2 (PLA_2) activity has been shown to be elevated in extracts of lesion-free psoriatic epidermis compared with extracts of normal epidermis.[1–3] This increased activity can be reduced following treatment with glucocorticoids both *in vivo*[4] and *in vitro*.[5] Glucocorticoids are thought to reduce PLA_2 activity through the production and release of an inhibitor[6] known as lipocortin. This inhibitor has been shown to be a protein of mol. wt. 40,000, the inhibitory activity of which is increased by dephosphorylation.[7]

By mixing extracts of lesion-free psoriatic epidermis with extracts of normal epidermis and demonstrating that the PLA_2 activity in the mixture is less than the sum of the components, we

Correspondence: Dr H.J.Yardley, Department of Biological Sciences, University of Keele, Staffordshire ST5 5BG, U.K.

have adduced that human epidermis contains an inhibitor of PLA_2.[2] If this inhibitor in epidermis is indeed lipocortin, any treatment calculated to remove phosphate groups from this molecule would be expected to reduce PLA_2 activity. Furthermore, protection of phosphate groups present in lipocortin from the action of endogenous protein phosphatases during the process of homogenization would be expected to increase PLA_2 activity.

METHODS

Materials

Radiochemicals were from Amersham International Plc (Little Chalfont, Bucks, U.K.). Calf intestinal alkaline phosphatase and lactate dehydrogenase attached to beaded agarose, agarose beads and phenyl methane sulphonyl fluoride were obtained from Sigma Chemical company Ltd (Poole, Dorset, U.K.). All reagents were of analytical grade.

Skin biopsies

Superficial skin biopsies (10 mm × 10 mm) were taken using a Davies dermatome (C.R.Thackray, Leeds U.K.) at setting 4, from 20 patients with psoriasis and 10 normal subjects, using ethyl chloride spray anaesthesia. In the psoriatics, the biopsies were at least 5 cm from the edge of the nearest lesion, from skin that was macroscopically normal. None of the subjects was on systemic therapy, nor had any used topical therapy in the 7 days prior to biopsy. Skin biopsies were taken with the approval of the North Staffordshire Health District Ethical Committee and the informed consent of the subjects.

Preparation of epidermal extracts

The skin samples were rinsed in 0·9% w/v sodium chloride, the epidermis was separated from the dermis using 2M potassium bromide and an epidermal extract was prepared as previously described[2] with the modification that the homogenizing medium contained 1 mmol/l phenyl methane sulphonyl fluoride to inhibit endogenous proteases. For some samples of epidermis, the homogenizing medium also contained sodium pyrophosphate at a final concentration of 30 mM. Protein was measured by the method of Lowry *et al.*[8]

Alkaline phosphatase treatment

An initial PLA_2 level was estimated in triplicate using 20 μl portions of epidermal extract as described previously.[2] A diluted suspension of insolubilized alkaline phosphatase was prepared by adding 1 volume of insolubilized alkaline phosphatase, as received from Sigma, to 24 volumes of 2M ammonium sulphate. A further 70 μl portion of each extract was incubated with 7 μl of the diluted suspension of insolubilized alkaline phosphatase for 30 min at 37°C; preliminary experiments had shown that longer incubation times did not further decrease the PLA_2 activity. Following this incubation, alkaline phosphatase was removed by centrifugation at 150 *g* for 3 min, after which PLA_2 was measured in triplicate in 20 μl portions of the supernatant. Controls were performed according to the same procedure using insolubilized lactate dehydrogenase, or agarose beads.

Statistical analysis

The results were found to be normally distributed as judged by a χ^2 test. Analysis was carried out using paired or unpaired Student's *t*-tests as appropriate.

RESULTS

Effect of treatment with alkaline phosphatase

Treatment of extracts of epidermis with insolubilized alkaline phosphatase significantly reduced the PLA$_2$ activity (Table 1). Treatment of epidermal extracts with insolubilized lactate dehydrogenase (Table 1) or agarose beads (data not shown), did not significantly reduce PLA$_2$ activity in any skin extract.

Effect of including pyrophosphate in the homogenizing medium

There was a very large increase in PLA$_2$ activity when the putative protein phosphatase inhibitor, pyrophosphate, was included in the homogenizing medium (Table 2). Extracts of

TABLE 1. Phospholipase A$_2$ (PLA$_2$) activity in extracts of normal and lesion-free psoriatic epidermis treated with insolubilized alkaline phosphatase or lactate dehydrogenase

	PLA$_2$ activity nmol fatty acid released/mg protein/h(mean ± SEM)	
	Normal epidermis	Lesion-free psoriatic epidermis
Initial activity	60·4 ± 4·4 (n = 10)	87·1 ± 6·9 (n = 20)*
Fall in activity after treatment with alkaline phosphatase	3·8 ± 1·4 (n = 10)**	16·1 ± 4·4 (n = 20)†
Fall in activity after treatment with lactate dehydrogenase	0·5 ± 2·6 (n = 4)	−0·8 ± 3·6 (n = 13)‡

* Significantly greater than normal epidermis. ($0{\cdot}01 < P < 0{\cdot}02$; unpaired t-test).

Mean fall in activity statistically significant, ** $P < 0{\cdot}02$.

† $0{\cdot}001 < P < 0{\cdot}002$ (paired t-tests).

‡ Negative value indicates an increase in activity.

TABLE 2. Effect of inclusion of pyrophosphate in the homogenizing medium on PLA$_2$ activity in extracts of normal and lesion-free psoriatic epidermis.

	PLA$_2$ activity nmol fatty acid released/mg protein/h (mean ± SEM)		
Added pyrophosphate	Normal epidermis	Lesion-free psoriatic epidermis	P
Absent	44·6 ± 3·9(n = 9)	91·5 ± 8·5(n = 13)	< 0·001
Present final concentration 30 mM	168·6 ± 19·7 (n = 9)*	170·5 ± 12·0 (n = 13)*	NS

* Significantly greater than extracts prepared in the absence of pyrophosphate; $P < 0{\cdot}001$.

normal and lesion-free psoriatic epidermis prepared in the absence of added pyrophosphate had significantly different PLA_2 activities. However, extracts of normal and lesion-free psoriatic epidermis prepared in the presence of pyrophosphate had almost the same high PLA_2 activity.

DISCUSSION

The mean PLA_2 activity in extracts of lesion-free psoriatic epidermis was significantly greater than that in normal epidermis. However, while the mean value for normal epidermis is similar to that previously reported,[2] the mean value for lesion-free psoriatic epidermis is considerably lower. This is because the PLA_2 activity in extracts of lesion-free psoriatic epidermis can extend over a wide range; in our present series of specimens, the values ranged from 48·6 to 154 units, with a preponderance of values at the lower end. Despite the large number of low values, a χ^2 test showed that the hypothesis that the values were normally distributed could not be rejected at the 95% confidence level.

The results of treating epidermal extracts with alkaline phosphatase are consistent with the hypothesis that the raised PLA_2 values seen in extracts of lesion-free psoriatic epidermis are caused by hyperphosphorylation of lipocortin. A variety of alkaline phosphatases, including preparations from calf intestine, has been shown to dephosphorylate phosphoproteins.[9] In order to exclude the possibility that PLA_2 was being removed by adsorption onto the enzyme-coated agarose beads, control experiments were performed using agarose beads coated with an enzyme unrelated to the phosphatases; when this was done, no reduction in PLA_2 activity was seen. Similar results were obtained using agarose beads alone (data not shown). It is important to note that we used calf intestinal alkaline phosphatase as a reagent to remove phosphate groups from proteins. We do not suggest that human epidermis contains an alkaline phosphatase; indeed, Mier and van Rennes[10] could barely detect alkaline phosphatase activity in human epidermis. However, a phosphotyrosyl phosphatase has been reported in mouse epidermis.[11]

The results following the inclusion of pyrophosphate in the homogenizing medium can be explained as follows. Pyrophosphate is a 'classical' protein phosphatase inhibitor[12] and we used it at a concentration which produces maximal inhibition.[13] In an extract of epidermis, one would expect to find both protein kinases and protein phosphatases. The relative activity of these two enzymes would determine the degree of phosphorylation of lipocortin, and hence the degree of inhibition of PLA_2. If protein phosphatases are inhibited, we would expect endogenous protein kinases to phosphorylate all available sites on lipocortin and render it minimally inhibitory to PLA_2. This accords with our observations that inclusion of a protein phosphatase inhibitor in the homogenizing medium raises the PLA_2 activity in extracts of normal and lesion-free psoriatic epidermis to the same high level. In the absence of added enzyme inhibitors in the homogenizing medium, we found PLA_2 activity in extracts of lesion-free psoriatic epidermis to be higher than that found in extracts of normal epidermis. We interpret this as meaning that in lesion-free psoriatic epidermis, compared with normal epidermis, protein kinase activity is high, or protein phosphatase activity is low, or both.

Our results with pyrophosphate did not indicate whether it was removal of phosphate groups from tyrosine, serine or threonine residues which was being inhibited, since pyrophosphate is a protein phosphatase inhibitor of broad specificity. However, although lipocortin can be phosphorylated on tyrosine and serine or threonine residues,[14] it seems likely that the anti-PLA_2 activity of lipocortin is controlled by phosphorylation of a tyrosine residue,[15] probably tyrosine 21.[16] Protein phosphorylation is a major mechanism for the regulation of the activity of enzymes, and a family of protein kinases which exhibit a novel specificity for tyrosine residues on target proteins has been linked to the regulation of cell proliferation in normal and transformed cells.[17] Members of this family of protein kinases include the receptors for epidermal growth factor and platelet-derived growth factor. As with all phosphorylation mechanisms, dephosphorylation by protein phosphatases is a necessary part of the regulatory system. Some protein phosphatases are regulated in turn by tyrosine protein kinases.[18]

In normal epidermis, phosphorylation of proteins on tyrosine is probably a relatively rare occurrence, as it is in untransformed cells.[19] We propose that in psoriatic epidermis, tyrosine residues are hyperphosphorylated. This would affect the activity of PLA_2 in two ways.

Firstly, lipocortin would have reduced PLA_2 inhibitory activity. Secondly, protein phosphatases might be rendered relatively inactive; this would spare phosphorylated lipocortin from dephosphorylation, thus allowing PLA_2 activity to remain high.

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Effect of a platelet release fraction on glycosaminoglycan synthesis by cultured dermal fibroblasts from patients with progressive systemic sclerosis

V. FALANGA AND STEPHANIE P. ALSTADT*

Department of Dermatology and Cutaneous Surgery, University of Miami, School of Medicine, Miami, Florida and
*Department of Dermatology, University of Pittsburgh School of Medicine Pittsburgh, Pennsylvania, U.S.A.

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SUMMARY

Confluent cultures of dermal fibroblasts from patients with progressive systemic sclerosis (PSS) and healthy controls were investigated for the synthesis of glycosaminoglycans (GAG) in response to a platelet release fraction (PRF) obtained by treatment of pooled platelets from normal individuals with adenosine 5′-diphosphate (ADP). PRF, at concentrations of 1 to 50 μg protein/ml, produced a linear increase in GAG synthesis that was always greater in cultures of PSS fibroblasts than in cultures of normal fibroblasts ($P < 0{\cdot}001$). A partial inhibition of GAG synthesis was observed with 100 μg/ml of PRF. The increased GAG synthesis in cultures incubated with PRF was not due to ADP. These findings demonstrate a difference in response to PRF between PSS and normal fibroblasts and may reflect an increased responsiveness of PSS fibroblasts to platelet factors.

Progressive systemic sclerosis (PSS) is a disease ultimately leading to fibrotic changes in the skin, blood vessels and internal organs from increased collagen deposition.[1] Glycosaminoglycans (GAG) are also produced in excess, especially in the early oedematous phase of the disease.[2] Raynaud's phenomenon and endothelial cell damage are considered the hallmark of PSS and frequently precede the other clinical manifestations. Therefore, a unifying hypothesis to explain the pathogenesis of PSS would favour a role for factors from plasma or platelets which, on diffusing into the dermis and other tissues from damaged capillaries, would stimulate fibroblasts and induce fibrosis. To date, investigators have described lymphokines and monokines that stimulate or inhibit fibroblast protein synthesis, including synthesis of collagen and GAG.[3–5] Well characterized and purified growth factors, including the platelet derived growth factors,

Correspondence: Dr Vincent Falanga, University of Miami School of Medicine, Department of Dermatology and Cutaneous Surgery, P.O. Box 016250, Miami, Florida 33101, U.S.A.

have failed to selectively stimulate DNA or collagen synthesis by PSS fibroblasts.[6] Other workers have shown a comparable increase in GAG synthesis by cultured PSS and normal fibroblasts in response to factors from lymphoid cells or platelets.[7]

We report here a study on GAG synthesis by PSS fibroblast cultures in response to a platelet release fraction (PRF) from healthy subjects.

METHODS

Patients

Dermal fibroblasts were obtained from three female patients (age range 37–50 years) with PSS fulfilling the criteria described by the American Rheumatism Association.[8] The patients all had active cutaneous disease and visceral involvement and had not received any treatment known to affect collagen metabolism. None had been treated with D-penicillamine, colchicine, systemic steroids, or other immunosuppresive agents. The disease duration was less than 1 year in all cases, measured from the time that proximal cutaneous induration was first recorded by a physician. After obtaining informed consent, a full thickness skin biopsy was taken from the dorsal aspect of the forearm, midway between the wrist and the elbow from an area of indurated and bound down skin. Biopsies were also obtained from the same site in three age and race matched healthy female volunteers.

Fibroblast cultures

Monolayer cultures of dermal fibroblasts were established from explant cultures of the dermal-subdermal portions of the biopsy specimens, which were minced and placed in 25 cm^2 plastic flasks (Corning). The tissue was allowed to air dry and attach to the plastic surface after standing at room temperature for approximately 10 min. At this time, 2 ml of nutrient medium consisting of Dulbecco's modified Eagle's medium (DME, Gibco Laboratories, Grand Island, NY, U.S.A.) supplemented with 10% fetal bovine serum (FBS, Gibco Laboratories), penicillin (100 μg/ml), and streptomycin (100 μg/ml), was gently added to the flask. Cultures were incubated at 37°C in 7% CO_2, 93% air. After outgrowth of cells from the explants (2 to 3 weeks), subcultures were established by trypsinization of the primary cultures. The fibroblasts were all in their second passage at the start of the experiments, and mycoplasma contamination was excluded in all cases (GenProbe, CA, U.S.A.).

Replicate monolayer cultures were established by inoculation of 2·1 cm^2 Nunc 4 well plates (Gibco Laboratories) with 20,000 cells/well on day 0. The medium was changed on days 3 and 5 with DME and 10% FBS. On day 7 the cultures were confluent and the medium in the experimental wells was replaced with 0·5 ml of DME and 3% FBS containing the factor being tested. The cultures were preincubated with the test factor for 48 h before the addition of ^{14}C-glucosamine.

Platelet release fraction

Pooled platelet concentrates were obtained from three normal volunteers. The platelets were washed by centrifuging at 2250 *g* for 15 min, and resuspending the platelet pellet in a platelet incubating medium (PIM) (100 mM Tris HCl, 100 mM KCl, 5 mM $MgCl_2$, 1 mM EDTA, pH 7·4). The suspension was centrifuged at 1,000 *g* for 5 min to remove remaining red cells and white cells. The platelet-rich supernatant was decanted and centrifuged at 5,000 *g* for 10 min, the PIM decanted, and the platelet pellet resuspended in DME. Red cell and white cell counts in the suspension were less than 10^5 cells/ml, and the platelet count was $1{\cdot}6 \times 10^9$ ml. Adenosine

5′-diphosphate (ADP; Sigma, St. Louis, MO, U.S.A.), 10 μM was added and incubated at 37°C for 15 min. Platelet aggregation and clumping on the walls of the plastic tube became evident within the first 2–3 min. Cell debris was removed by centrifugation at 16,000 *g* for 15 min and the resultant platelet release fraction (PRF) was sterilized using a Millex 0·45 μm filter. Protein concentration was determined by a colorimetric assay.[9]

Measurement of glycosaminoglycans

On day 9, 48 h after the addition of PRF, monolayer cultures were pulsed with 0·4 μCi of ^{14}C-glucosamine (New England Nuclear) for 20 h. Then 0·1 ml of DME containing 5 mg/ml pronase (Calbiochem-Behring Corporation, San Diego, CA, U.S.A.) was added to each well. The fibroblasts detached and cell numbers were determined using a haemocytometer. The remaining cell suspension (0·4 ml) containing both cells and medium, was incubated for 3 h at 50°C and centrifuged to clear. Carrier GAG, consisting of 0·1 ml of a solution of hyaluronic acid (1 mg/ml) and chondroitin sulphate (1 mg/ml) (Sigma, St. Louis, MO, U.S.A.), was then added to each sample followed by 1·0 ml of 1% cetylpyridinium chloride (CPC) in 0·002 M $NaSO_4$ to precipitate the GAG.[10] The precipitates were collected on Whatman GF/F glass fibre filters (Whatman Inc., Clifton, NJ, U.S.A.). Each filter was washed twice with 0·1% CPC in 0·5 M NaCl and four times with distilled water. They were placed in liquid scintillation counting vials and dried for 20 h in an oven at 45°C. Scintillation fluid (ACS, Amersham) was then added and ^{14}C measured by a Tracor Analytic scintillation counter (Model 6891, Elk Grove Village, IL, U.S.A.). The results obtained with this method for measuring GAG have been shown to reflect GAG synthesis determined by a modified carbazole (Eastman Kodak) technique for uronic acid.[11]

Statistical analysis

Student's *t*-test was used to analyse the results of GAG measurements.

RESULTS

The effect of platelet release fraction on GAG synthesis

In initial experiments we used a maximum PRF concentration of 100 μg protein/ml. The effect of 20 or 100 μg/ml of PRF on GAG synthesis by cultured fibroblasts from the PSS patients and the controls is shown in Figure 1. The addition of 20 μg/ml of PRF caused up to a four fold increase in GAG synthesis relative to baseline. This increase in GAG synthesis was significantly greater in PSS fibroblasts than in normal fibroblasts ($P < 0{\cdot}001$). In both PSS and normal fibroblasts, PRF, at 100 μg/ml, stimulated GAG synthesis compared with baseline ($P < 0{\cdot}01$) but the effect was less than that seen with 20 μg/ml of PRF ($P < 0{\cdot}001$). There was no significant difference in GAG synthesis between PSS and normal fibroblasts with 100 μg/ml PRF.

The effect of increasing concentrations of PRF on GAG synthesis by cultured fibroblasts from two PSS patients and two controls is shown in Figure 2. Increasing the PRF concentration from 1 to 50 μg/ml gave a linear increase in GAG synthesis ($r = 0{\cdot}905$ and 0·987 for PSS and normal fibroblasts, respectively). Although baseline GAG synthesis was the same in PSS and normal fibroblasts, the addition of PRF always led to greater GAG synthesis in PSS fibroblast cultures than in the control cultures ($P < 0{\cdot}001$). Fibroblast counts at the end of the experiment were not significantly different in PSS and normal cultures. For example, representative cell counts (mean ± SD) after the addition of 20 μg/ml PRF were $27{\cdot}4 \pm 3{\cdot}7 \times 10^4$ for PSS cultures and $24{\cdot}1 \pm 4{\cdot}4 \times 10^4$ for normal cultures, compared with $27{\cdot}2 \pm 4{\cdot}1 \times 10^4$ and $21{\cdot}1 \pm 3{\cdot}9 \times 10^4$,

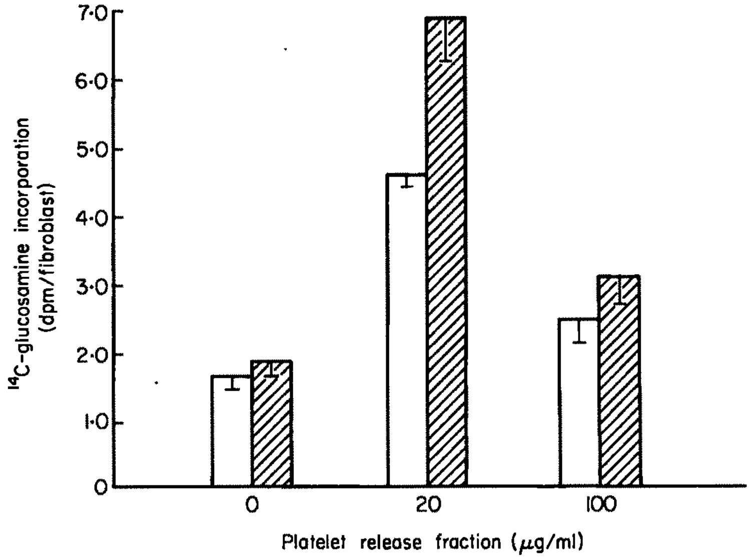

FIGURE 1. The effect of platelet release fraction on GAG synthesis. Results are means ± SEM of determinations from quadruplicate wells of fibroblasts from three PSS patients (▨) and three normal controls (□).

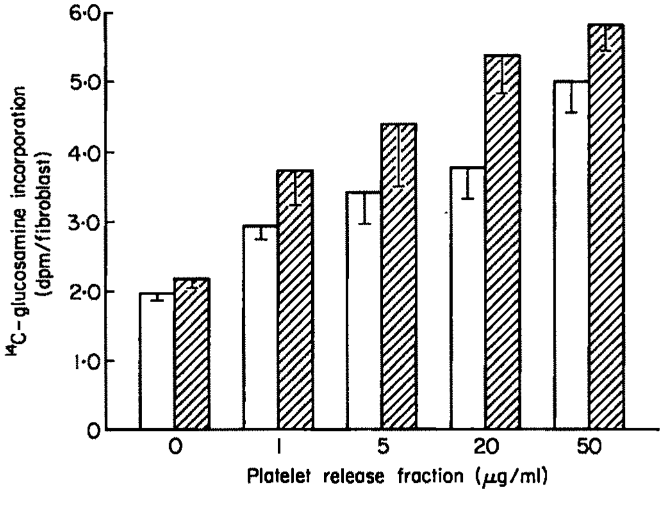

FIGURE 2. The effect of increasing PRF concentrations on GAG synthesis by PSS (▨) and normal (□) fibroblasts. Results are means ± SEM of determinations from quadruplicate wells of fibroblasts from two PSS patients and two normal controls.

respectively for cultures without the addition of PRF ($P > 0{\cdot}1$). Thus PRF did not preferentially affect the proliferation of either PSS or normal fibroblasts.

The GAG-stimulatory activity of PRF was not affected by freezing in liquid nitrogen for 24 h, but was completely abolished after freezing for 2 weeks (data not shown).

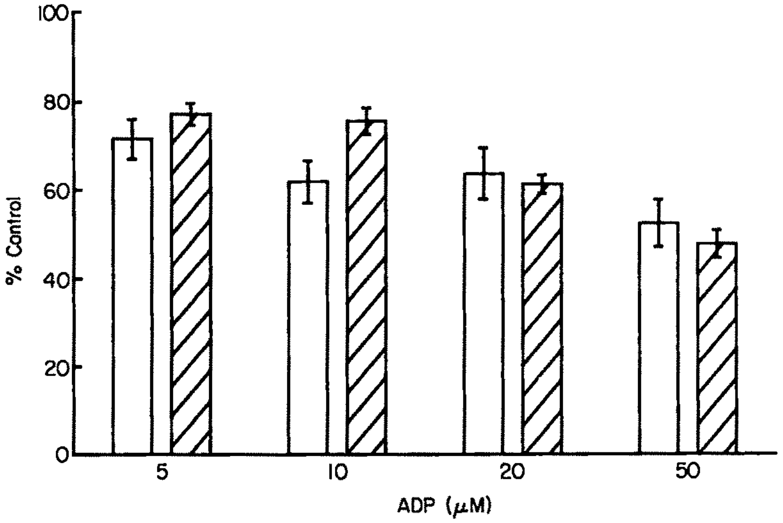

FIGURE 3. The effect of increasing ADP concentrations on GAG synthesis by PSS (▨) and normal (☐) fibroblasts. Results are the mean ± SEM of duplicate determinations from triplicate wells.

Effect of ADP on GAG synthesis

We performed additional experiments to ensure that the ADP used to initiate platelet aggregation and release did not, itself, stimulate GAG synthesis. The effect of increasing ADP concentrations on GAG synthesis by cultured fibroblasts are shown in Figure 3. Increasing the ADP concentration caused a linear decrease in GAG synthesis by both PSS and normal fibroblasts ($r = -0{\cdot}898$ and $-0{\cdot}736$, respectively).

In separate experiments we found that increasing the concentration of ADP added to the platelets from 10 μM to 100 μM did not result in a greater protein yield of PRF or greater GAG-stimulatory activity by the PRF (data not shown).

DISCUSSION

In the present study we have shown that an ADP-induced platelet release fraction prepared from pooled platelets from normal volunteers increased GAG synthesis by confluent cultures of human dermal fibroblasts. This extends our previous more limited observation using a crude platelet fraction in fibroblast cultures from one patient with PSS.[12] PRF produced a larger increase in GAG synthesis by PSS fibroblasts than by normal fibroblasts. These findings suggest that factors released from platelets upon aggregation may modulate the synthetic activity of fibroblasts and play an important role in the excessive accumulation of connective tissue that occurs in PSS. The role of platelet factors in normal fibroblast proliferation and metabolism was reported some time ago.[13]

We do not know the reason for the loss of GAG-stimulatory activity of the PRF preparation after storage in liquid nitrogen for several days. This information might prove useful for identifying specific platelet factors responsible for the effect of PRF on PSS fibroblasts. We have found that transforming growth factor-beta (TGF-beta), which is stored in platelets, had a greater GAG-stimulatory effect on PSS fibroblasts than on normal fibroblasts.[14] However, although TGF-beta is stable for many months at acid pH at −20°C, we have not studied its effect on GAG synthesis after prolonged storage in liquid nitrogen.

Our findings may be important since various platelet factors acting together may be necessary for certain biological activities,[15] and ADP-induced platelet aggregation and subsequent secretion is known to occur *in vivo*.[16] Various hypotheses might help to explain our findings. It is possible that *in vivo* exposure of PSS fibroblasts to platelet factors leads to a clonal expansion of fibroblasts with a higher synthetic response to platelet factors that persists *in vitro*. Changes in PSS fibroblasts other than a clonal expansion, such as perhaps increased receptor levels for platelet growth factors, might also explain our results. Alternatively, there may be *in vivo* or *in vitro* factors other than platelets that may play an indirect but important role in making PSS fibroblasts more responsive to PRF.

There are possible mechanisms by which dermal fibroblasts in PSS might be exposed to an abnormally sustained action of platelet factors. Endothelial cell damage occurs early in the skin of PSS patients.[17,18] This endothelial cell damage, consisting of gaps, vacuolization, and necrosis, could lead to platelet aggregation and diffusion of released platelet factors into the surrounding dermis. Moreover, there is evidence that platelets from PSS patients may aggregate more easily.[19] The finding of elevated plasma levels of beta-thromboglobulin in PSS may be further evidence that increased platelet secretion may occur in PSS.[20] Thus, a pathogenetic role of platelet factors in PSS could arise through damage to endothelial cells and more easily activated platelets.

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A new elastic system component, 'elastic sheet' in the human dermis. An electron microscopic study

T. TSUJI

Department of Dermatology, Osaka City University Medical School, Osaka, Japan

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SUMMARY

We have studied a new component of the elastic fibre system in the human dermis, which we call 'elastic sheet', using scanning and transmission electron microscopy. The structure is long, broad and flat with some holes, and may be the base of the elastic fibre system from which elastic fibres originate.

It is now generally accepted that there are elastic system fibres consisting of oxytalan, elaunin and elastic fibres in the human dermis.[1,2] The oxytalan fibres are present in the upper dermis or adjacent to the follicular epithelium and arise from a plexus of elaunin fibres. Elaunin fibres are connected with the elastic fibres in the reticular dermis. Ultrastructurally, the oxytalan fibres appear as a bundle of microfibrils similar to elastic microfibrils, but without amorphous material. The elaunin fibres present as a dispersed amorphous material intermingled with the microfibrils. The elastic fibres have amorphous material in the centre and microfibrils at the periphery.

In addition to these three components of elastic system fibres, scanning electron microscopic studies have disclosed the presence of a new component which we have called 'elastic sheet', in the human dermis. The purpose of the present study was to characterize this elastic sheet in normal skin and in scars and striae distensae.

METHODS

Normal skin specimens were taken from the thigh of three children (aged 2 to 5 years), five young adults (aged 18 to 38 years) and four elderly individuals (aged 66 to 81 years). Specimens of scar tissue or striae distensae were obtained from various parts of the body in six patients (aged 6 to 45 years). The age of the scar or striae ranged from 1 month to 20 years. The specimens

Correspondence: Takuo Tsuji, Department of Dermatology, Osaka City University Medical School, 1-5-7 Asahimachi, Abeno, Osaka 545, Japan.

were processed for scanning electron microscopy (SEM) and transmission electron microscopy (TEM).

For scanning electron microscopy, the method was based upon that described previously.[3] The specimens, approximately 1 mm thick, were placed in distilled water and autoclaved for 8 h at 121°C and a pressure of 18 lb/in^2. The autoclaved specimens were fixed at 4°C in 2% glutaraldehyde in 0·1 M phosphate buffer at pH 7·4 for 1 h, then rinsed in the buffer for 1 h. After dehydration in a graded series of ethanols (70–100%), the samples were placed in 100% xylol for 2 h and air dried. Dried samples were mounted on aluminium stubs using cellophane tape. They were coated with a thin layer of gold palladium in a sputter coater and viewed in a Hitachi S-800 scanning electron microscope operated at 5–15 kV.

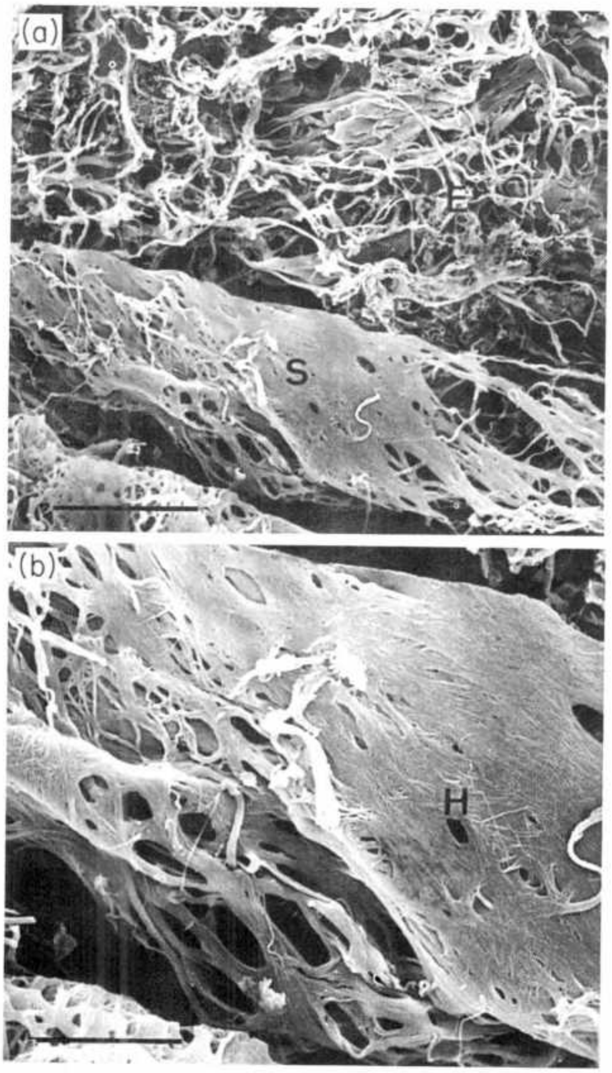

FIGURE 1. SEM of normal skin from a 25 year-old man. a) dermal elastic fibres (E) and an elastic sheet (S), a long, broad and flat structure. (Bar = 50 μm) (b) Higher magnification. The surface is relatively smooth with some holes (H). (Bar = 20 μm).

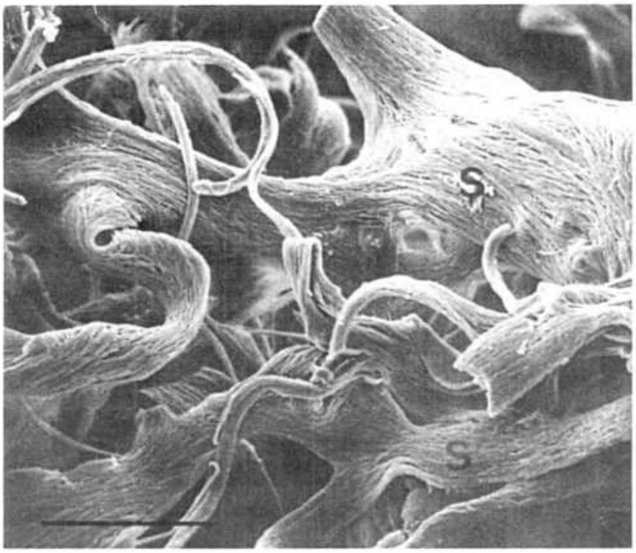

FIGURE 2. SEM of normal skin from a 66 year old man, showing some elastic sheets (S). The surface is relatively rough, consisting of a number of thinner fibrils aligned parallel to the main axis of the sheet. (Bar = 20 μm).

For transmission electron microscopy, the specimens were fixed at 4°C for 1 h in 2% glutaraldehyde, with 0·2 M phosphate buffer (pH 7·4). After thorough rinsing they were immersed in 1% osmium tetroxide with 0·2 M phosphate buffer (pH 7·4) for 1 h and then dehydrated in a graded ethanol series and embedded in Epon 812. Ultrathin sections were cut with a Porter-Blum MT-2 ultra-microtome. Some sections were stained with uranyl-acetate for 1 h and with Reynold's lead citrate for 10 min. Others were stained with tannic acid–uranyl acetate solution[4] for 10 min and with Reynold's lead citrate for 10 min. They were examined with a Hitachi H-300 transmission electron microscope.

The width and length of the sheet-like structure of elastic fibres in normal specimens from the three age groups and in the scars or striae were measured on the scanning electron micrographs using an appropriate scale.

RESULTS

Scanning electron microscopy

In the specimens from the children and the young adults, a sheet-like structure of elastic fibres was seen only in the deep dermis, together with normal-shaped elastic fibres (Fig. 1a, b). This was more often seen in the young adults than in the children. In the specimens from the elderly individuals, the number of these structures was increased, and they were seen not only in the deep dermis, but also in the middle and even in the upper dermis (Fig. 2). In some old scars and striae distensae (more than 10 years old), the sheet structure was irregularly distributed in the deep or middle dermis. It often ran straight and parallel to the skin surface and appeared to be much wider and longer than that found in normal skin from any age group (Fig. 3a).

The width and length of the sheet-like structure in normal skin from the three age groups, and in scars and striae are presented in Table 1. The sheet-like structure was significantly wider and

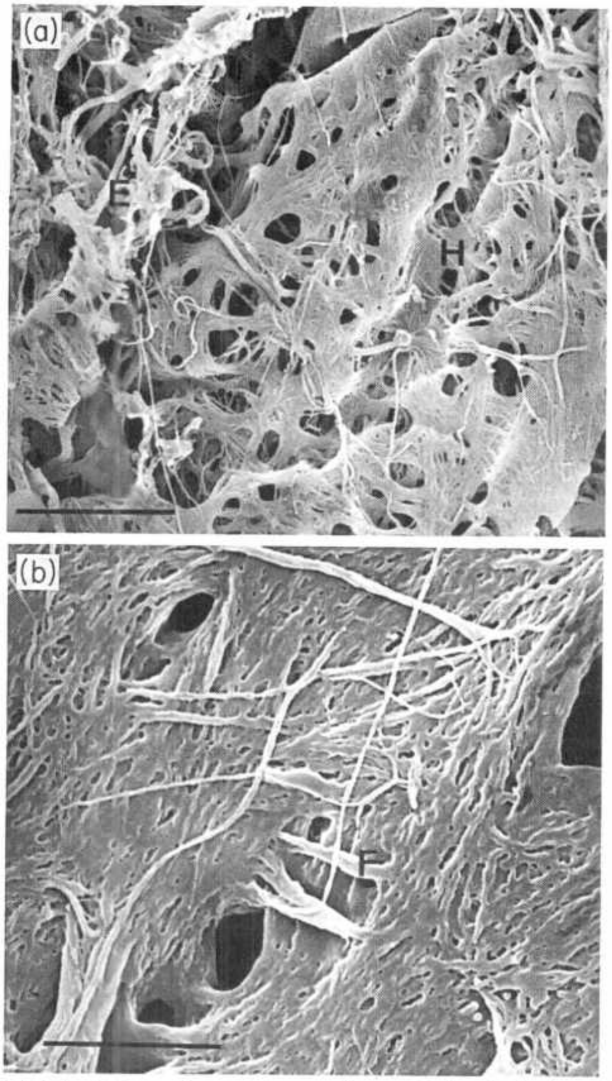

FIGURE 3. SEM of striae distensae from a 23 year-old man. (a) An elastic sheet showing a broad irregular structure with many holes (H). E = elastic fibre. (Bar = 20 μm). (b) Higher magnification showing finer fibrils (F) forming bridges, not only with adjacent fibrils, but also with fibrils further away. (Bar = 6μm).

longer in the specimens from the young adults and the elderly individuals than in those from the children ($P < 0{\cdot}01$) and in the specimens from the scars or striae distensae than in normal skin ($P < 0{\cdot}001$). However, there was no significant difference in the width and length in the young adults and the elderly individuals, although the width in the latter tended to be less.

The surface of the sheet-like structure was relatively smooth and had few holes in the children's specimens. It was more irregular and had more holes in specimens from older subjects, and in the scars and striae. The surface consisted of a number of thinner fibrils (0·2 to 1·0 μm diameter) aligned parallel to the main axis of the sheet, forming bridges with adjacent

TABLE 1. The width and length of elastic sheets in normal skin from individuals of three age groups and in old scars or striae

	Width (μm)		Length (μm)	
Group	Range	Mean ± SD	Range	Mean ± SD
Children	6–9	7 ± 2 ($n = 7$)	10–50	30 ± 11 ($n = 7$)
Young adults	10–25	$15 \pm 4^{*}$ ($n = 12$)	30–70	$48 \pm 17^{*}$ ($n = 10$)
Elderly individuals	7–20	$12 \pm 3^{*}$ ($n = 19$)	25–65	$42 \pm 26^{*}$ ($n = 10$)
Old scars or striae distensae	40–120	$73 \pm 20^{**}$ ($n = 5$)	250–400	$320 \pm 68^{**}$ ($n = 4$)

* Significantly different from the value in the children ($P < 0{\cdot}01$; Student's t-test).

** Significantly different from normal skin ($P < 0{\cdot}001$; Student's t-test).

fibrils (Fig. 2). Furrows between the fibrils outlined these structures. The structure branched into several finer fibres at the periphery, some being continuous with the elastic fibres surrounding it, others recombining and forming the sheet. In the old scars and striae, the thinner fibrils often formed bridges, not only with adjacent fibrils, but also with fibrils further away (Fig. 3b).

Transmission electron microscopy

The sheet-like structure consisted of amorphous material and microfibrils similar to that seen in elastic fibres. It differed from the elastic fibres in that the structure was much broader and had several holes in the sheet, corresponding to those seen on SEM (Fig. 4). Neither vessels nor

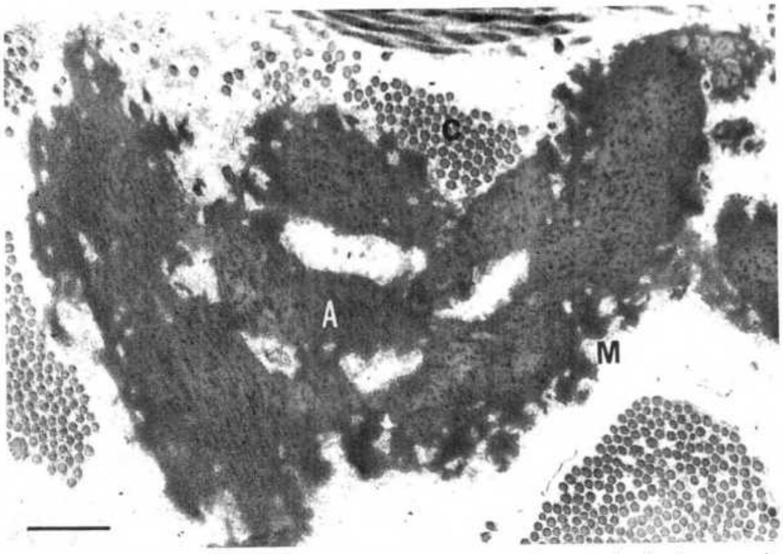

FIGURE 4. TEM of normal skin from a 30 year-old man, showing an elastic sheet consisting of amorphous material (A) and microfibrils (M). The structure is broad with some holes which do not contain any cells or tissue. C = collagen fibres. (Tannic acid stain; Bar = 1 μm).

collagen fibres were seen in the holes. There were no substantial differences in the ultrastructure of the structures found in normal skin and in scars or striae distensae.

DISCUSSION

The present study has demonstrated the consistent presence of a sheet-like structure of elastic fibres mainly in the deep dermis of normal skin and some old scars and striae distensae. It is characteristically a long, broad (6 to 25 μm in width) and flat structure with some holes, while normal elastic fibres measure 0·5 to 3·5 μm in diameter and have no holes.[5] To our knowledge, there has been no previous description of such a sheet-like structure in the elastic system fibres.[1,2] It is probable that previous investigators did not find this structure because they used light microscopy and TEM, but not SEM. We suggest this structure be called 'elastic sheet' and we regard the structure as the fourth component of the elastic fibre system, in addition to oxytalan, elaunin and elastic fibres.

The exact function or action of this sheet is unclear. It may be the base of the elastic fibre system from which elastic fibres originate, and may prevent hyperextension of the skin on stretching because the elastic fibres are fixed to it. Elderly skin is known to be less elastic than young skin, and scars and striae are known to be harder and less extensible than normal tissue. This could be related to the presence of more sheets in older skin and to the presence of much larger sheets in old scars and striae.

A similar structure to the elastic sheet is present in the walls of the aorta and other arteries, and is called 'elastic lamina'.[6,7] The fenestra in the elastic lamina may be analogous to the holes in the elastic sheet. However, these structures differ in that endothelial cells and smooth muscle cells form communicating links through the fenestra of the elastic lamina, while no cells or tissues form communications through the holes in the elastic sheet.

The reason why the elastic sheets in old scars and striae distensae were longer and broader than those in normal skin is unclear. The sheet-like structure was only seen in scars and striae distensae more than 10 years old. It seems that the size of the sheet may increase very slowly with the age of the scars or striae. In our previous study on scars, we found that elastic fibres were newly synthesized, and then increased and became thicker as the scars got older, although their shape and distribution pattern were very irregular.[8] A similar process may be occurring in the case of elastic sheets.

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Non-lymphoid accessory cells in the cutaneous infiltrate of B cell lymphomas. An immunohistochemical and ultrastructural study

N. PIMPINELLI, P. ROMAGNOLI*, S. MORETTI AND B. GIANNOTTI

Second Dermatological Clinic and *Department of Human Anatomy and Histology, Section of Histology 'Enrico Allara', University of Florence, Italy

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SUMMARY

We have investigated the occurrence, immunohistochemical profile, ultrastructural features and relationships to lymphocytes of the non-lymphoid accessory cells in the dermal infiltrate of five patients affected by B cell lymphoma with secondary involvement of the skin. Typical non-lymphoid accessory cells were found in all cases. Most of these cells had ultrastructural features which resembled those of the poorly differentiated dendritic reticulum cells described in follicular lymphomas of the lymph nodes. The immunohistochemical findings of DRC-1+, C3b r+ dendritic cells often arranged in follicular-like structures with neoplastic B cells and only few, scattered OKM1+, OKM5+ mononuclear phagocytes support the hypothesis that the vast majority of the non-lymphoid cells observed in our cases were poorly differentiated dendritic reticulum cells.

These results and previously published reports indicate that the organization of the dermal infiltrate of B cell lymphomas tends to reproduce the typical arrangement of the B zone of the lymphoid tissue, although with a lesser degree of differentiation, similar to that observed in lymph node follicular lymphomas.

In normal lymphoid tissue, lymphocytes interact with other cells types, stromal cells and accessory cells.[1,2] Stromal cells are regularly associated with collagen bundles and basal membrane-like material; these cells form the reticular framework of the lymphoid tissue, dividing it into compartments and hence, directing migrating lymphocytes and accessory cells. Accessory cells interact with T and B lymphocytes during acquired specific immune responses; they can also mediate non-acquired, 'natural' immunity independently of lymphocytes.

In the germinal centres of secondary lymphoid follicles, which are specific sites of B cell differentiation, typical accessory cells, dendritic reticulum cells, are found.[3–6] Dendritic reticulum cells exhibit 5-nucleotidase activity,[7] bear complement (C_3) receptors on their

Correspondence: Dr Nicola Pimpinelli, Clinica Dermatologica II, Via della Pergola 58, 50121, Firenze, Italy.

surfaces[8] and specifically react with R4/23 monoclonal antibody.[9] On electron microscopy, these cells characteristically show narrow plasma membrane infoldings coated with electron dense material, presumably representing fixed immunocomplexes; this hypothesis is supported by autoradiographic[3] and immunohistological studies.[8,10] Dendritic reticulum cells thus appear to play a role in trapping antigen within germinal centres. They also form a specific microenvironment that is preserved in low-grade malignant lymphomas, of which follicular lymphoma is the most common example.[11–15]

Dendritic reticulum cells have been identified in the cutaneous infiltrate of B cell lymphomas by enzyme-cytochemistry and immunohistochemistry.[16–20] Little is known about the ultrastructure of non-lymphoid accessory cells in cutaneous B cell lymphomas; some authors have observed, in some cases of cutaneous B cell lymphoma, non-lymphoid cells with the features of either dendritic reticulum cells or fibroblastic reticulum cells.[21–23] Further investigations are necessary to assess the extent of the participation of these cells in the cutaneous infiltrate of B cell lymphomas and to investigate their ultrastructural features and their relationships with infiltrating neoplastic lymphocytes. Better knowledge on these points is necessary before it is possible to speculate on their significance and their possible functional role (if any) in these lymphomas.

We report here the occurrence, immunohistochemical profile, ultrastructural features and relationship to lymphocytes of non-lymphoid accessory cells in the dermal infiltrate of five patients with B cell lymphoma with secondary involvement of the skin.

METHODS

Five Caucasian patients, seen in the Dermatological Clinic, University of Florence, were studied. Before starting treatment, a skin biopsy was taken from each patient and processed for light and electron microscopy and for immunohistochemisty. Clinical details of the patients are summarized in Table 1. The diagnosis was made according to the Kiel classification.[5,24]

Light microscopy

The specimens were fixed in phosphate buffered formol and routinely embedded in paraplast. Histological sections were stained with haematoxylin and eosin, PAS, and reticulin stain.

Immunohistochemistry

The tissue was snap-frozen and stored at −80°C. Cryostat sections (6 μm) were air dried, fixed

TABLE 1. Clinical details of patients studied

Patient no.	Age years	Sex	Cutaneous lesions	Primary localization (lymph nodes)	Diagnosis (type of lymphoma)
1	80	F	Multiple nodules (trunk, abdomen, extremities)	Right axillary	CB, diffuse
2	20	M	Solitary plaque (trunk)	Mediastinal	IB, diffuse
3	68	M	Solitary nodule (scalp)	Right laterocervical	CB/CC, diffuse
4	56	M	Solitary plaque (lower abdomen)	Left inguinal	CC, diffuse
5	22	M	Solitary plaque (trunk)	Left laterocervical	CB, diffuse

CB = centroblastic; CB/CC = centroblastic/centrocytic; CC = centrocytic; IB = immunoblastic.

in chloroform-acetone for 5 min and incubated for 30 min with the primary monoclonal antibody. The monoclonal antibodies used are listed in Table 2. The sections were then incubated with biotin-conjugated sheep anti-mouse serum (Amersham, U.K.) for 30 min and with streptavidin-biotin-peroxidase complex (Amersham, U.K.) for 20 min. The staining reaction was obtained with aminoethylcarbazole and hydrogen peroxide. The sections were finally counterstained with Mayer's haematoxylin and mounted with glycerine. Normal human lymph nodes served as positive controls. Sections incubated without the primary monoclonal antibody or without any antibody were negative controls for the second polyclonal antibody and for the peroxidase enzyme reaction, respectively. For quantitative analysis, the stained cells were counted in five consecutive microscopic fields at high magnification (×400). The mean number of cells was calculated. Only cells in which the nucleus was in the plane of the section were considered.

Electron microscopy

Skin biopsies were fixed in 4% glutaraldehyde in 0.1 M cacodylate buffer, pH 7·3, postfixed in 1% OsO_4 in 0.1 M phosphate buffer, pH 7·3, and embedded in Epon 812. Ultrathin sections were stained with uranyl acetate and lead citrate and observed with a Siemens Elmiskop 1 or 102 electron microscope at 80 kV.

RESULTS

Immunohistochemistry

In all cases, B cells (Leu 12+, HLA-DR+) made up the majority of the infiltrating cells. In Case 2, a kappa light chain monoclonality was observed; in the other cases the dermal infiltrate did not react with anti-kappa or anti-lambda light chain antibodies.

A variable proportion of infiltrating cells (higher in Cases 3 and 4 than in the others) reacted with OKT11, OKT4 and OKT8 monoclonal antibodies, the T4/T8 ratio ranging between 1:2 and 2:1.

DRC-1+, C3b r+ cells with a dendritic shape were consistently observed in the neoplastic infiltrate. In all cases except one (Case 2), these cells were gathered into follicular-like clusters with Leu 12+ lymphocytes (Figs 1, 2 and 3); the centre of the clusters contained mainly IgM+ cells, the peripheral rim contained mainly IgD+ cells. These clusters were separated from each

TABLE 2. Monoclonal antibodies used in the study

Antibody	Commercial source	Specificity
Leu 12	Becton and Dickinson, USA	
anti-HLA-DR	"	
anti-kappa	"	
anti-lambda	"	
anti-IgM	"	
anti-IgD	"	
OKT11	Ortho Diagnostic Systems, USA	see Foon & Todd, 1986[25]
OKT4	"	
OKT8	"	
OKM1	"	
OKM5	"	
anti-DRC 1	Dakopatts, Denmark	
anti-C3b receptor	"	

other by areas containing both Leu 12+ cells and reactive T11+ T lymphocytes, but devoid of DRC-1+, C3br+ cells. In Case 2, DRC-1+, C3br+ cells were scattered among the infiltrating neoplastic cells without any follicular-like organization.

Some OKM1+, OKM5+ cells were dispersed among the infiltrating cells.

Electron microscopy

In all the patients, the dermal infiltrate mainly consisted of lymphocytes and typical non-lymphoid cells.

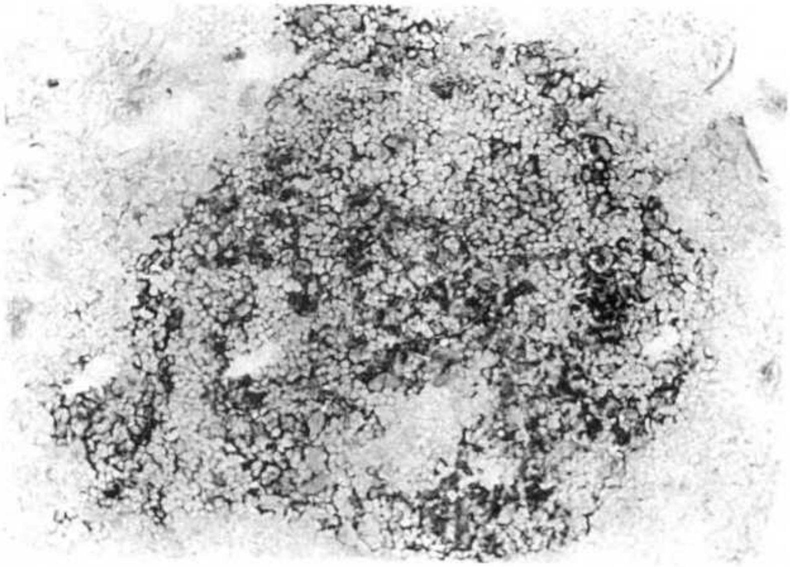

FIGURE 1. DRC-1+ cells collected into a follicular-like structure with B cells (Case 3). (Original × 250).

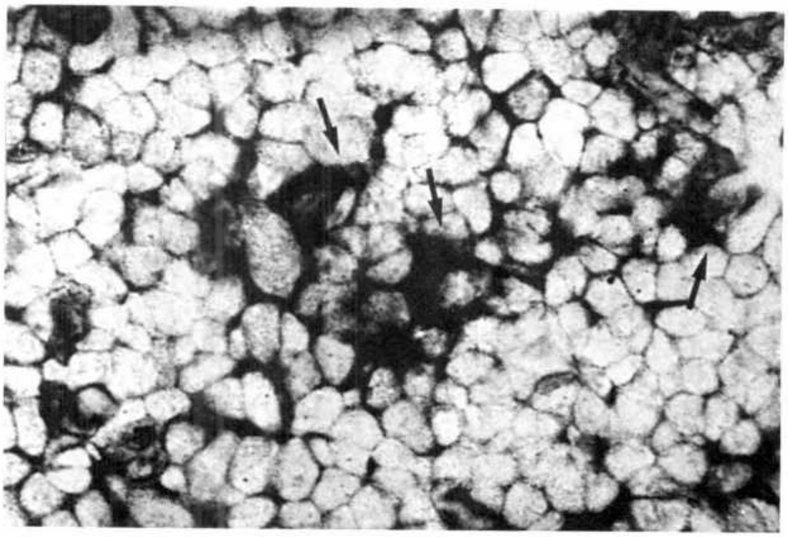

FIGURE 2. The dendritic shape of some DRC-1+ cells (arrows) is clearly shown (Case 3). (Original × 700).

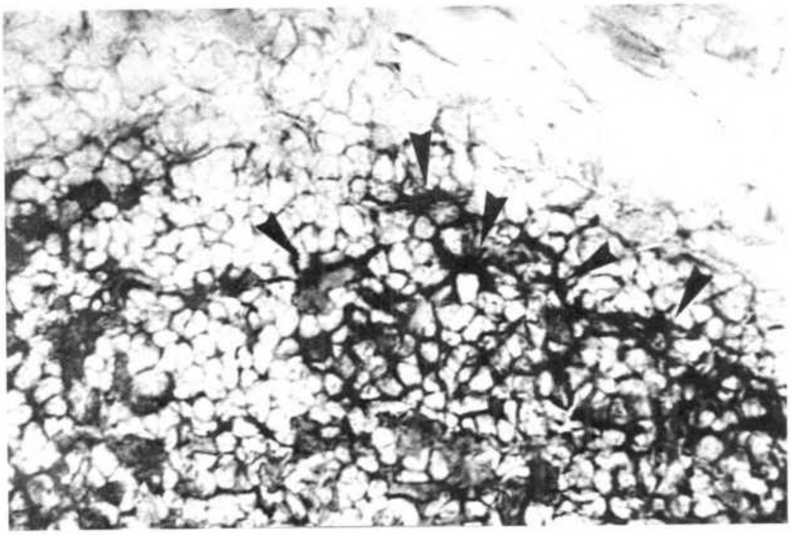

FIGURE 3. C3br+ dendritic cells (arrowheads) with weakly stained B cells (Case 4). (Original × 450).

Most of the non-lymphoid cells had an irregular shape, with cytoplasmic projections which varied in number and length in different patients. The plasma membrane formed a small number of narrow invaginations (Fig. 4), which were not coated with electron dense material. Desmosome-like junctions between these cells were rare. The cytoplasm of these cells contained

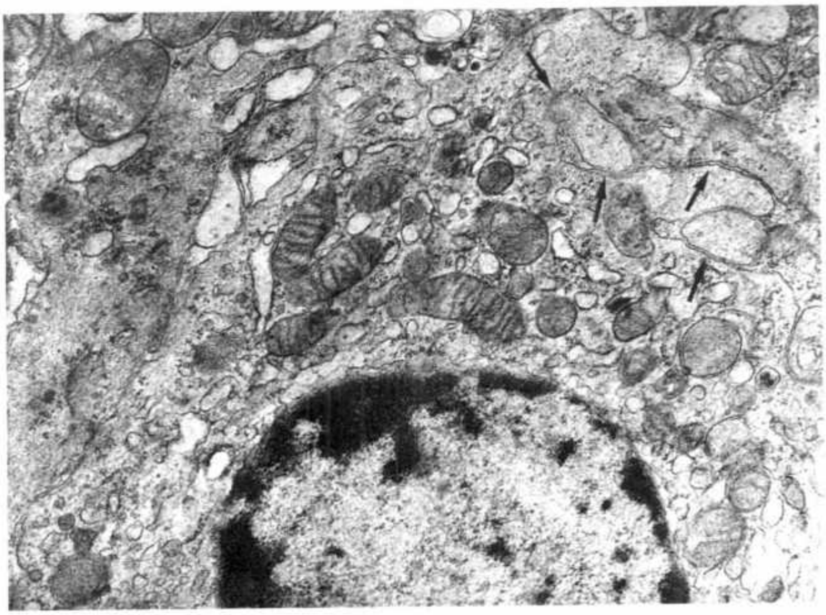

FIGURE 4. Non lymphoid cell in the dermal infiltrate of Case 1. Many narrow invaginations of the plasma membrane are shown (arrows). The cytoplasm contains small mitochondria, few cisternae of rough endoplasmic reticulum and many smooth surfaced vesicles and tubules. A rim of microfilaments is located beneath the plasma membrane (original × 27,000).

flat cisternae of rough endoplasmic reticulum, a well developed Golgi apparatus (Fig. 5), smooth vesicles and tubules (Fig. 6), some coated vesicles close to the plasma membrane (Fig. 5) and a variable number of round, membrane bound bodies, presumably primary lysosomes (Fig. 6). Secondary lysosomes or residual bodies were rare. The nucleus (Fig. 5) had a frequently indented outline, with a peripheral rim of condensed chromatin, a usually well recognizable fibrous lamina and a small nucleolus. Few of these cells were binucleated, or perhaps multinucleated (Fig. 7). These non-lymphoid cells and their cytoplasmic projections always contacted several lymphocytes (Figs 5 and 6).

Some non-lymphoid cells had few, slender cytoplasmic processes which extended among collagen bundles. The cytoplasm of these cells (Fig. 8) contained several cisternae of rough endoplasmic reticulum, a Golgi apparatus less developed than in the above mentioned cells, few Golgi vesicles and no smooth vesicles or tubules outside the Golgi region; coated vesicles and round, homogeneously dense, membrane bound bodies were virtually absent. These cells always had a single nucleus, with a more condensed chromatin pattern than in the cells mentioned above.

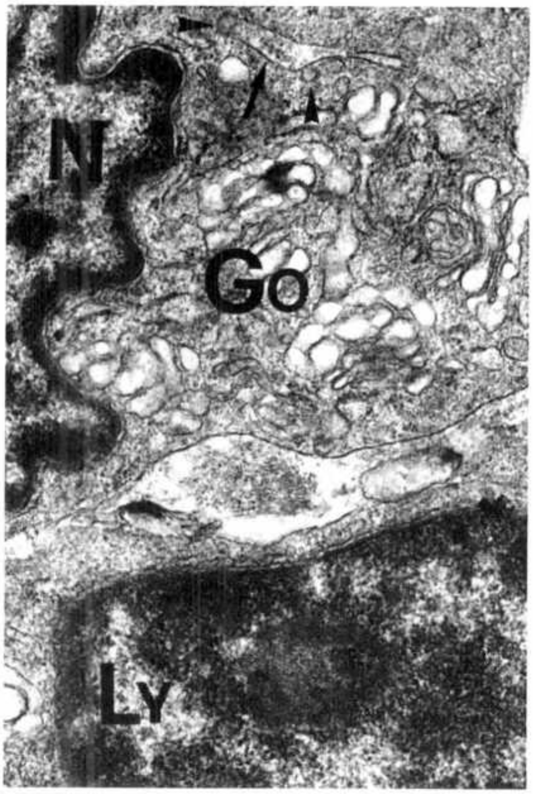

FIGURE 5. Non lymphoid cell in the dermal infiltrate of Case 5. The nucleus (N) has a slightly, but definitely, indented outline with a thin peripheral rim of condensed chromatin and a well recognizable fibrous lamina. In the cytoplasm, the Golgi apparatus (Go) is well developed. A cross-sectioned invagination of the plasma membrane (arrow), with two coated pits (arrowheads), is also shown. This cell is in contact with an infiltrating lymphoid cell (Ly), identification of which was made at low magnification. (Original × 27,000).

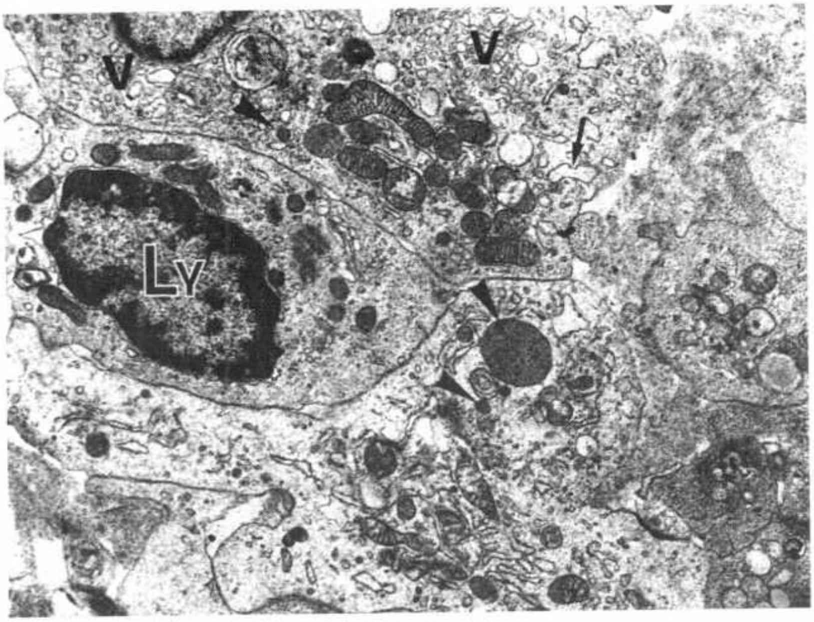

FIGURE 6. Dermal infiltrate from Case 4. A lymphoid cell (Ly) is in contact with two non lymphoid cells. The cytoplasm of the latter cells contains some mitochondria and cisternae of rough endoplasmic reticulum, numerous smooth surfaced vesicles (V) and tubules (arrows) and few, round, homogeneously dense bodies of different size (arrowheads), presumably primary lysosomes. (Original × 11,500).

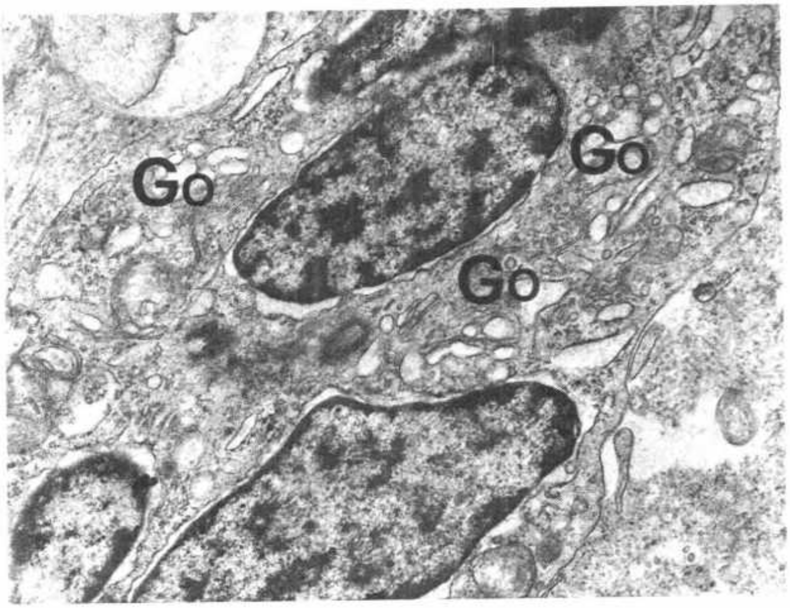

FIGURE 7. Dermal infiltrate from Case 3. A non lymphoid cell with extensive Golgi apparatus (Go) and scanty cisternae of rough endoplasmic reticulum contains four nuclear profiles, possibly pertaining to 2–4 nuclei. (Original × 16,000).

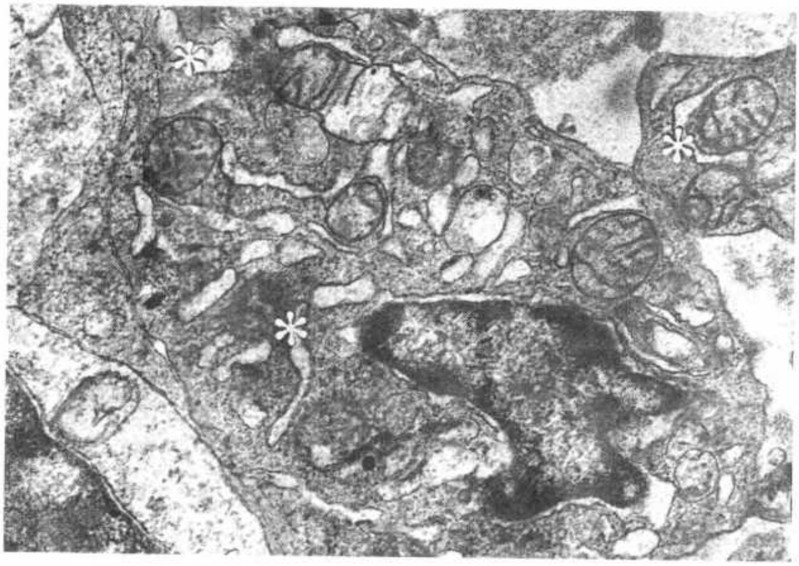

FIGURE 8. Dermal infiltrate from Case 4. A non lymphoid cell is shown, which contains several cisternae of rough endoplasmic reticulum (asterisks). This cell type is different from that shown in Figures 4–7, and resembles fibroblasts. The Golgi apparatus and smooth vesicles and tubules are inconspicuous in this cell type (Original × 13,000).

DISCUSSION

Typical non-lymphoid cells were found in all of five cases of secondary cutaneous B cell lymphoma.

Relatively few of these cells resembled the fibroblastic reticulum cells (stromal cells) described in normal lymphoid tissue[1,2,6] and in cutaneous B cell lymphomas.[21–23] They had few, slender cytoplasmic processes extending among collagen bundles; the cytoplasm contained several cisternae of rough endoplasmic reticulum, a poorly developed Golgi apparatus, few Golgi vesicles and no smooth vesicles or tubules outside the Golgi region; coated vesicles and round, homogeneously dense, membrane bound bodies were not seen; these cells always had a single nucleus, with a moderately condensed chromatin pattern.

Most of the non-lymphoid cells observed in our cases had an irregular shape, more or less dendritic, and shared some ultrastructural features with dendritic reticulum cells described in the germinal centres of secondary lymphoid follicles.[1–6] More precisely, these cells had plasma membrane invaginations, cytoplasmic organization, nuclear outline, chromatin pattern and relationships with lymphocytes similar to dendritic reticulum cells, albeit the invaginations of their surfaces were seldom extensively labyrinthine, electron dense material did not coat the plasma membrane and, as a rule, desmosome-like junctions between these cells and hemidesmosome-like junctions between them and the intercellular matrix were absent. These ultrastructural features suggest that these cells are poorly differentiated dendritic reticulum cells, also on account of the ultrastructure of dendritic reticulum cells described in follicular lymphomas of lymph nodes.[14,15] In follicular lymphoma of lymph nodes, in comparison with reactive follicular hyperplasia, dendritic reticulum cells usually have broader cell processes and sometimes less developed cytoplasmic organelles. Also, in follicular lymphomas of lymph nodes, binucleated dendritic reticulum cells are rare, desmosome-like junctions between adjoining cells are relatively uncommon and labyrinthine plasma membrane infoldings are never coated with electron dense material. However, in follicular lymphomas of lymph nodes, histiocytic cells are significantly less numerous than in reactive hyperplasia and show no pronounced phagocytic activity, either on light[5] or electron microscopic examination,[14,26] so that the ultrastructural differentiation between histiocytic and dendritic reticulum cells may be a very difficult task.

Comparing our ultrastructural findings with the immunohistochemical finding of DRC-1 +, C3br + dendritic cells often arranged with neoplastic B cells in follicular-like structures and few, dispersed, OKM1 +, OKM5 + cells, which have also been found previously in B cell lymphoma, both in lymph nodes[13] and in the skin[18–20] we interpret the vast majority of the non-lymphoid cells observed in our cases as poorly differentiated dendritic reticulum cells. The follicular-like arrangement of lymphoid and non-lymphoid cells, which was also found in areas without any morphological evidence of lymphoid follicles, reinforces the interpretation of most of the non-lymphoid cells as dendritic reticulum cells.

Our present results and reports in the literature,[16–23] suggest that skin infiltrating neoplastic B cells induce the development of a specialized microenvironment consisting of non-lymphoid B zone-specific accessory cells, albeit with a limited degree of differentiation, in a similar way to that observed in lymph node follicular lymphomas.[5,11–15]

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Ro/SSA antigen in human epidermis

S.K.JONES, SILVIJA COULTER, CATHERINE HARMON,* D.A.NORRIS, MARY ROSANDICH,* AND LELA A.LEE

Departments of Dermatology and *Medicine, University of Colorado School of Medicine and Veterans' Administration Hospital, Denver, Colorado, U.S.A.

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SUMMARY

It has been shown previously by an immunofluorescence technique, that whole serum from patients who have anti-Ro/SSA autoantibodies reacts with a component or components of the epidermis. We have now demonstrated by immunoblotting that the antigen identified in human epidermis by anti-Ro/SSA sera is Ro/SSA antigen, and that Ro/SSA antigen is present both in adult and in neonatal epidermis. The presence of this antigen in tissues which are injured in the anti-Ro/SSA-associated syndromes subacute cutaneous lupus erythematosus and neonatal lupus erythematosus supports the hypothesis that anti-Ro/SSA antibodies react with Ro/SSA antigen in the skin and are important in the initiation of tissue damage.

The cutaneous lupus syndromes, subacute cutaneous lupus erythematosus (SCLE) and neonatal lupus erythematosus (NLE) are characterized by photosensitivity, a non-scarring papulosquamous eruption, and in the case of NLE, congenital heart block.[1–4] Serologically, they show a strong association with the presence of anti-Ro/SSA autoantibodies, these being found in 82% of patients with SCLE[2] and about 95% of NLE patients.[3] Although antigen Ro[5] and SSA antigen[6] were described separately, subsequent collaborative studies have shown them to be immunologically identical.[7] The antigen, therefore, is referred to here as Ro antigen. Whether anti-Ro antibodies are involved in the pathogenesis of SCLE or NLE is still open to debate. It is important to know, when considering the possible importance of these autoantibodies in disease, if the antigen to which they are directed is present in relevant target tissues such as adult and neonatal skin.

The first evidence for Ro antigen in skin was found by LeFeber *et al.*[8] who reported the reactivity of cultured neonatal keratinocytes with anti-Ro sera using immunofluorescence (IF) techniques. The anti-Ro sera gave a particulate (speckled) pattern on cultured keratinocyte cell nuclei and cytoplasm. After ultraviolet irradiation of the cells, augmentation of antibody binding was seen on the cell surfaces.

Lee *et al.*[9] examined normal fetal tissues and normal neonatal and adult epidermis for the presence of Ro antigen by IF and by precipitation in agarose, either by counter immunoelectro-

Correspondence: Lela A.Lee, University of Colorado School of Medicine, Box B-153, 4200 East Ninth Avenue, Denver, Colorado, U.S.A. 80262.

phoresis (CIE) or by Ouchterlony immunodiffusion. Ro antigen was found, by IF and by CIE or immunodiffusion, to be present in neonatal epidermis, adult epidermis, and all fetal internal organs examined, including fetal heart.

Lee *et al.*[10] then showed that adult human skin grafted onto nude mice bound IgG from injected anti-Ro sera. Subsequent studies have demonstrated that purified anti-Ro antibodies bound to the human skin graft (Lee *et al.*, unpublished) The adjacent mouse skin, which did not contain Ro antigen as detected by IF, showed no antibody binding. This provided further evidence that the Ro antigen is indeed present in normal adult human epidermis, and is present on the cell surface where it is accessible for antibody binding. Subsequently we reported,[11] that the injection of anti-Ro serum into the guinea pig (an animal that has Ro antigen at detectable levels) resulted in the deposition of antibody in the skin and heart in a pattern similar to that found in SCLE and NLE patients.[10,12]

These results appear to support the hypothesis that anti-Ro antibodies may be involved in the pathogenesis of SCLE and NLE. However, other investigators[13] have not been able to confirm all our findings, principally that of the presence of Ro antigen in adult human skin. Furthermore, with the technique of IF and CIE or immunodiffusion we cannot exclude the possibility that the anti-Ro sera reacted with an antigen crossreacting with Ro or that a different antibody was present in the anti-Ro sera which was reacting with an antigen other than Ro. To establish more conclusively that it is actually Ro that is being recognized in epidermal preparations, we examined epidermal extracts for the presence of Ro antigen by sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE) and immunoblotting.

Tissue preparations

Adult epidermis was obtained from skin removed during abdominoplasty or mammoplasty, and neonatal epidermis was obtained from circumcisions. About 130 cm^2 of skin was used to prepare adult extract, and approximately 25 foreskins were used to prepare neonatal extract. Subcutaneous fat was trimmed from the skin; then the skin was placed in phosphate buffered saline (PBS), pH 7·2 with protease inhibitors, and stored at −70°C. Protease inhibitors were 1 mM phenylmethylsulfonyl fluoride (PMSF) 5 mM N-ethyl maleimide, and 5 mM EDTA, pH 7·4. The skin was thawed, cut into 3 mm^2 pieces, and incubated in 2·5 mg/ml collagenase at 37°C for 2 h. The epidermis was peeled from the dermis and placed in PBS with protease inhibitors. The epidermis was mechanically disrupted using a ground glass tissue homogenizer, sonicated (using six 15 s bursts), and centrifuged at 7500 *g*, at 4°C for 1 h. The supernatant was decanted and concentrated in an Amicon concentrator (Danvers, MA, U.S.A.; molecular weight cut-off: 10 kd). The adult extract had a final protein concentration of 6·4 mg/ml, and the neonatal extract had a final protein concentration of 11 mg/ml, as measured by the Bradford dye binding assay (BioRad; Richmond, CA, U.S.A.).

Antisera

Anti-Ro serum was obtained from 10 subjects who had subacute cutaneous lupus erythematosus or who were mothers of babies with neonatal lupus erythematosus. Four sera were found to be monospecific for anti-Ro antibodies by immunodiffusion analysis. The remainder had anti-Ro and other autoantibodies (usually anti-La). The antigens detected by these additional autoantibodies were of distinctly different molecular weights from Ro antigen, as determined by immunoblotting. Anti-Ro titres were measured in these sera by CIE; the titres ranged from 1:64 to 1:256. Control serum was obtained from nine healthy volunteers; these sera were antinuclear antibody (ANA) negative.

Immunoblotting

Epidermal extract samples were diluted in sample buffer containing Tris-HCl, glycerol, sodium dodecylsulphate (SDS) 2-beta mercaptoethanol and bromophenol blue. Dilutions of extract in sample buffer were approximately 1:2 for adult epidermis and 1:4 for neonatal epidermis. After boiling for 3 min, samples were loaded onto an SDS 10% polyacrylamide gel 16 cm long and 1·5 mm thick at 0·08 mg protein/lane and 0·025 ml loading volume/lane. Electrophoresis was carried out at 30 mA for 6 h in a 4°C environment. Proteins were transferred to 0·45 μm pore diameter nitrocellulose paper (BioRad; Richmond, CA, U.S.A.) overnight at 40 V in transblot buffer containing methanol, Tris, and glycine. The nitrocellulose strips were placed in blocking buffer (Tris-buffered saline (TBS) with 3% gelatin) for 45 min. This and all subsequent incubations were carried out at room temperature in plastic sealable bags (Dazey; Industrial Airport, KS, U.S.A.). After incubations with blocking buffer and antibody solutions, the nitrocellulose strips were washed in TBS with 0·05% Tween-20, and then placed in antisera diluted 1:100 in antibody buffer (TBS with 1% gelatin) for 2 h. The strips were then incubated with horseradish peroxidase-protein A (BioRad) diluted 1:2500 in antibody buffer, for 1 h. Colour development was carried out using 4-chloro-1-napthol (BioRad).

RESULTS

All 10 anti-Ro sera identified a protein of molecular weight 60 kd in both adult and neonatal epidermis. All anti-Ro sera identified this protein on several occasions. In all, 23 immunoblots were run, seven blots with adult epidermis only, four with neonatal only, and 12 with adult and neonatal epidermis. None of the nine control sera used in these blots identified the 60 kd protein or any other epidermal protein. A representative immunoblot is shown in Figure 1.

In the 12 blots which were run comparing directly adult and neonatal epidermis, there were no quantitative differences that could be detected by this technique between the 60 kd protein in adult epidermis and in neonatal epidermis. The slight difference in intensity of colour development between adult and neonatal epidermis as shown in Figure 1 was not a consistent finding. In some blots, the adult 60 kd band appeared slightly more intense. In most cases, there was no discernable difference between adult and neonatal epidermis.

With only one exception, the intensity of staining for the 60 kd protein was closely related to the titre of anti-Ro antibody detected by CIE. There was no apparent relationship between the titre of anti-Ro antibody in an individual patient's serum, as measured by immunoblotting or CIE, and the clinical findings in the patient.

DISCUSSION

Ro antigen is a protein of molecular weight 60 kd that is generally found in a 100 kd complex with RNAs.[14] Neither the function of Ro antigen nor that of the antigen-RNA complex is known.[14,15] So far, antigenicity has been detected only in the 60 kd protein portion of the complex. When gels are run under denaturing, reducing conditions, as in the present experiments, the 60 kd protein rather than the 100 kd protein-RNA complex is identified by anti-Ro sera.[14]

Our finding of a 60 kd protein identified by 10 different anti-Ro sera and by none of nine control sera is evidence that Ro antigen is present in human epidermis. The titres of anti-Ro in CIE were closely related to the intensity of the colour on immunoblotting, with all but one serum, which is further evidence that the 60 kd protein is Ro.

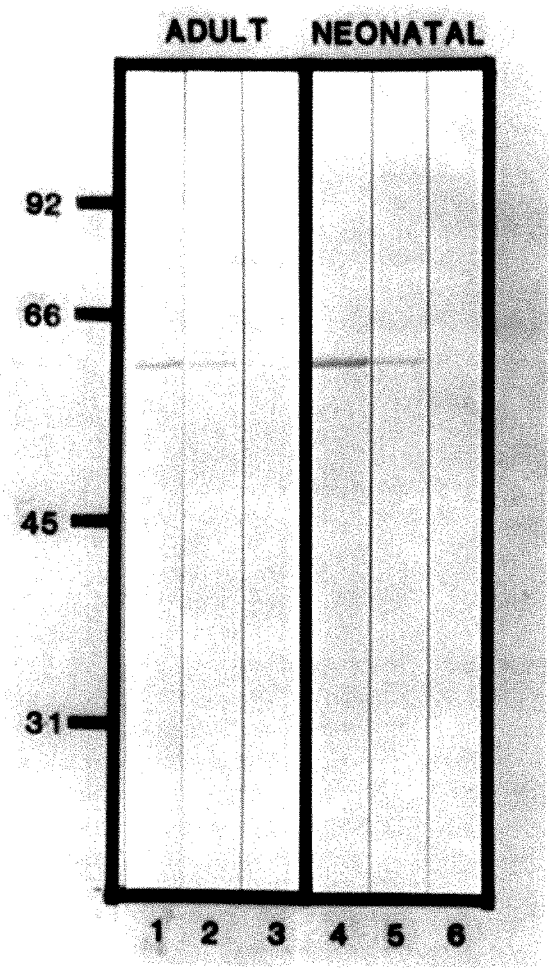

FIGURE 1. Immunoblot of adult and neonatal epidermal extracts reacted with anti-Ro sera and normal serum and separated by SDS-PAGE. Lanes 1–3: adult epidermal extract. Lanes 4–6: neonatal epidermal extract separated via SDS-PAGE. The migration positions of standard molecular weight markers are shown on the left. Lanes 1 and 4 were reacted with anti-Ro serum 1, lanes 2 and 5 with anti-Ro serum 2, and lanes 3 and 6 with normal serum.

These results verify our previous impression that Ro antigen is present in the epidermis, both in adults and in neonates. In our experience, it is very important to concentrate the epidermal extracts and then to run a sufficiently large amount of total protein (about 0·080 mg protein/lane for a gel 16 cm long and 1·5 mm thick). At a total protein content of 0·040–0·060 mg/lane, Ro could generally not be detected (results not shown). Furthermore, the immunoblotting conditions described here represent the optimal conditions out of many tested. Since Ro is a protein present in relatively low amounts in the cell,[15] selecting optimal conditions is important for this protein to be detectable.

The present findings lend further support to the hypothesis that anti-Ro antibodies bind to Ro antigen in the skin in subacute cutaneous lupus erythematosus and neonatal lupus erythematosus and may play an important role in tissue damage in these diseases.

ACKNOWLEDGMENTS

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Immunohistological detection of interleukin 1-like molecules and tumour necrosis factor in human epidermis before and after UVB-irradiation *in vivo*

ANNEMETTE OXHOLM*‡, P.OXHOLM†‡, B.STABERG*, AND K.BENDTZEN†

*Department of Dermatology, Gentofte Hospital, †Laboratory of Medical Immunology 7544, Rigshospitalet, University Hospital, ‡Pathological-anatomical Institute, Kommunehospitalet, Copenhagen, Denmark.

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SUMMARY

Skin biopsies from five healthy subjects, taken before and after UVB irradiation, were examined using immunohistological techniques for the cytokines interleukin-1 (IL-1) and tumour necrosis factor (TNF). Using polyclonal specific antibodies against IL-1 and TNF, the two cytokines appeared identically located on the epidermal cell membranes of the stratum granulosum and stratum spinosum in unexposed skin. After UVB-exposure, the staining intensity for both IL-1/epidermal cell derived thymocyte-activating factor (ETAF) and TNF was markedly increased, and the epidermal staining included the basal cell layer.

Immunohistological investigation of tissue-bound epidermal cytokines may be valuable in the study of skin diseases.

Increasing evidence indicates that epidermal cells are capable of releasing immuno-regulatory factors,[1] and epidermal cells may actively participate in initiation and regulation of immune responses. These immuno-regulatory factors, or cytokines, are usually examined *in vitro* by bioassays or *in vivo* in animal models.

Recent evidence suggests that activated epidermal cells produce a molecule which is closely related to macrophage-derived interleukin-1 (IL-1). This cytokine is termed epidermal cell derived thymocyte-activating factor (ETAF).[1] Like IL-1, ETAF causes a number of local and systemic inflammatory and immunological reactions,[1] and ETAF may, therefore, participate in immune reactions in normal skin.

Tumour necrosis factor (TNF) was so named because the molecule induced haemorrhagic

Correspondence: Annemette Oxholm, MD, Department of Dermatology, Gentofte Hospital, Niels Andersensvej 65, DK-2900 Hellerup, Denmark.

necrosis in certain animal tumours and in heterotransplanted human tumours.[2,3] It appears to be cytotoxic also for tumour cell lines.[4] TNF has been found in the serum of BCG/endotoxin-treated mice,[2,3] and in serum and spinal fluids from patients with meningococcal sepsis (Bendtzen *et al.* unpublished findings). The major cellular origin of TNF seems to be endotoxin-stimulated macrophages and monocytes.[3,5]

Intermediate wavelength ultraviolet radiation (UVB) is an environmental factor that influences the immunoreactivity of the skin as well as the entire body.[6]

The object of the present investigation was to examine whether IL-1/ETAF and TNF could be detected in normal human epidermis by an immunohistological technique. We also investigated the influence of UVB on the location and quantity of these cytokines within the epidermis.

METHODS

Subjects

Studies were performed in five fair-skinned, healthy, informed volunteers (aged 26 to 39 years) who were not receiving any medication.

Cytokines

Human recombinant pI 7 IL-1 (rIL-1β) (produced by Cistron Biotechnology, Inc. Pine Brook, NJ, USA) was a kind gift from Dr. Dinarello (New England Medical Center, Boston, MA, USA). This IL-1 consists of amino acids 112-269 of the precursor sequence.[7] Human recombinant pI 5 IL-1 (rIL-1α) was kindly donated by Immunex (Seattle, WA, U.S.A.), and human recombinant TNF (rTNF) was provided by the Boehringer-Institute (Vienna, Austria). Natural monocyte IL-1 (molecular size 17·5 kd) (nIL-1) was donated by Dr Dinarello, who produced and purified the mediator from *Staphylococcus albus*-stimulated blood adherent cells, as previously described.[8]

Production of specific antisera to IL-1, nIL-1 and TNF

The antiserum raised against purified human blood monocyte-derived nIL-1 was produced and purified by absorption with human AB serum and blood leukocyte supernatants devoid of IL-1 activity as described previously.[9] This antiserum to nIL-1 does not react with interleukin-2, interferon-γ, lymphotoxin or TNF (Bendtzen, unpublished data).

The specific antisera to human rIL-1α, rIL-1β and rTNF were produced and kindly donated by Dr Dinarello. Rabbits were immunized with 10 intradermal injections on the back with 2% fresh glutaraldehyde plus 100 μg of carrier-free recombinant-derived, purified antigen in complete Freund's adjuvant with 1 mg dried *Mycobacterium butyricum*. After 1 month, the animals were boosted every week with 1 μg of antigen intravenously, and every month with 50 μg of antigen intramuscularly in incomplete Freund's adjuvant. After 6 months the different antisera were tested, and those rabbits with neutralizing antibodies (titres greater than 1:100) were boosted for 3 more months. Judged by Western blot analysis, there was no cross-reactivity between rIL-1α, rIL-1β and rTNF, and their respective antisera.[10] There was also no cross-reactivity when testing these antisera for direct neutralization of rTNF- and IL-1 activities, and no measurable cross-reactivity was detected between the specific anti-rIL-1α and anti-rIL-1β antisera (Dinarello and Bendtzen, unpublished results).

Irradiation procedure

The UVB source was a bank of two fluorescent sunlamps (Phillips TL 20W/12), emitting in the

270-380 nm range, with a maximum at 310 nm. The irradiance was measured by an IL 700 research radiometer with detector PT 171 C/297 (International Light Inc.).

The individual minimal erythema dose (MED) was determined by irradiation of nine 1·5 × 1·5 cm square test sites on untanned gluteal skin with a geometric series of 40% increments of exposure dose. The UV tubes were adjusted to 18 cm above the skin surface. At this distance the irradiance in the UVB range (280–315 nm) was 0·9 mW/cm^2. The MED was defined as the lowest radiation dose required to produce a faint and well defined erythema 24 h after exposure. After determination of MED on one buttock, the irradiation was carried out with three MED on the other buttock before punch biopsy.

In all subjects the UVB radiation caused distinct erythemal responses. The mean MED (± SD) was 184·4 ± 67·7 mJ/cm^2.

Skin biopsies

Punch biopsies (3 mm) of unexposed and irradiated skin were obtained simultaneously 24 h after irradiation, under local anaesthesia produced by infiltration of 2% lidocaine in a ring around the biopsy site. The tissue was snap-frozen and stored at −70°C.

Biotin-avidin technique for demonstration of in vivo-bound epidermal IL-1/ETAF and TNF

Sections of the skin specimens, 4–6 μm thick, were cut in a cryostat. The sections were air dried for 5 min, fixed in acetone for 1 min, and then incubated for 5 min with 3% hydrogen peroxide in distilled water followed by repeated washing in Tris buffer (0·05M Tris/HCl, pH 7·6) for 5 min. The sections were then incubated for 20 min with 20% normal swine serum (Dakopatts, Copenhagen, Denmark) in Tris buffer in a humidity chamber. Excess serum was removed, and sections incubated for 30 min at 29°C with diluted anti-IL-1 or anti-TNF antiserum, using two-fold dilutions in the range 1:10–1:640. The sections were then washed three times for 5 min in Tris buffer followed by incubation for 30 min at 20°C with biotinylated swine anti-rabbit immunoglobulin (Dakopatts) diluted 1:300 in Tris buffer. After washing three times in Tris buffer for 5 min, the sections were incubated with AB Complex (Dakopatts) for 30 min at 20°C and washed as before. The sections were then incubated with 0·038% 3-amino-9-ethylcarbazol (Sigma, St. Louis, U.S.A.) and 0·014% hydrogen peroxide for 5 min, washed in water for 10 min, counterstained with haematoxylin for 2½ min, and finally washed in water for 10 min. The samples were mounted with Gurr aquamount (BDH chemicals, Poole, Dorset, U.K.).

Biotin-avidin technique for demonstration of OKT6-positive epidermal Langerhans cells

The mouse monoclonal antibody OKT6 (Ortho Diagnostic Systems, New Jersey, U.S.A.) was used at a 1:100 dilution as the primary layer antibody. Biotinylated rabbit anti-mouse immunoglobulin (Dakopatts) was the secondary layer antibody at a 1:300 dilution. The sections were otherwise treated as described above.

Staining of adjacent sections of biopsies taken before and after irradiation with UVB was performed in parallel in order to compare the cellular location of the cytokine positive cells and the OKT6-positive epidermal Langerhans cells.

Immunohistological controls

The anti-nIL-1 antiserum was diluted in 10% normal human serum in Tris buffer because this was shown to decrease the presumed non-specific cytoplasmic staining. This procedure did not change the membrane staining. Using anti-TNF antiserum, the staining was not affected by addition of human serum, and this antiserum was therefore diluted in Tris buffer alone.

The specific staining was lost if either the primary antibodies (anti-cytokine antibodies) or the secondary layer antibodies were omitted, or if the anti-cytokine antibodies were replaced by Tris buffer or normal rabbit serum (Dakopatts).

Pre-incubation of the anti-nIL-1 antiserum (1 μ1 diluted 1:40) with rIL-1α (10–5000 units) or rIL-1β (10–5000 units) for 30 min at 20°C did not alter the staining with this antiserum. Pre-incubation with a combination of the two species of rIL-1 also did not alter the staining. On the other hand, pre-incubation for 30 min at 20°C of 10^5 units rTNF with 1 μl anti-rTNF antiserum (diluted 1:40) abolished staining by this antiserum, whereas 500 units rTNF failed to alter the staining caused by the anti-nIL-1 antiserum. Pre-incubation for 30 min at 20°C of 500 units of nIL-1 with 1 μl anti-rTNF antiserum (diluted 1:40) or with 1 μl anti-nIL-1 antiserum (diluted 1:40) did not modify the staining by these antisera.

RESULTS

Anti-nIL-1 and anti-rTNF staining patterns in normal skin

Very similar staining patterns were obtained with the two different anti-cytokine antibodies, and only minor differences were observed between skin samples from the five subjects. Granular staining of the intercellular spaces of the epidermis was found in all five specimens. Staining was localized either to the membranes of single cells or to groups of cells predominantly found in the stratum granulosum and stratum spinosum (Figs 1a, 2a and 3). On light microscopic examination it was not possible to determine whether the surface of cell membranes or structures in the intercellular spaces stained. Epidermal cell membrane staining was not seen in the basal cell layer. Laminar staining of the stratum corneum was also evident. Cytoplasmic staining was only seen when high concentrations of antibodies were used, and only in cells with membrane staining. Accessory skin structures were demonstrated in a few biopsies. Hair follicles stained similarly to the epidermis, whereas sebaceous glands and sweat gland ducts did not stain. Table 1 shows the maximum dilutions of primary layer antibodies which produced staining of epidermal cell membranes.

Staining patterns in UVB-irradiated skin

The two different antisera stained the epidermis in a topographically identical fashion. However, in contrast to non-irradiated skin, the membrane staining now included the basal cell layer (Figs 1b and 2b). Also, the staining intensity was increased, and in several cases further dilution of the primary layer antibodies still gave staining (Table 1).

Staining with OKT6 antibodies

The staining for cytokines was found in areas with and without OKT6-positive cells. It was not possible to determine whether the cytokine staining of the spaces surrounding the OKT6-positive cells was confined to the membranes of Langerhans cells or whether the membranes of neighbouring keratinocytes were stained.

DISCUSSION

The immunohistological technique used in the present study was successful in demonstrating the presence of human TNF and, possibly, IL-1/ETAF in normal and UVB-irradiated skin. This conclusion is based upon the use of polyclonal, but highly specific, antisera against these

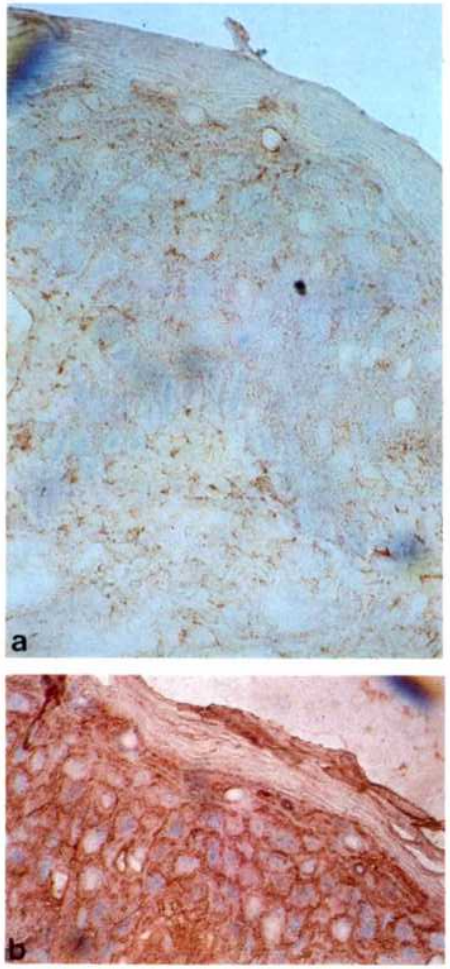

FIGURE 1 Epidermal *in vivo*-bound IL-1/ETAF demonstrated using a biotin-avidin-peroxidase technique (original × 800). (a) Unexposed skin from a normal subject. (b) UVB-irradiated skin from the same subject.

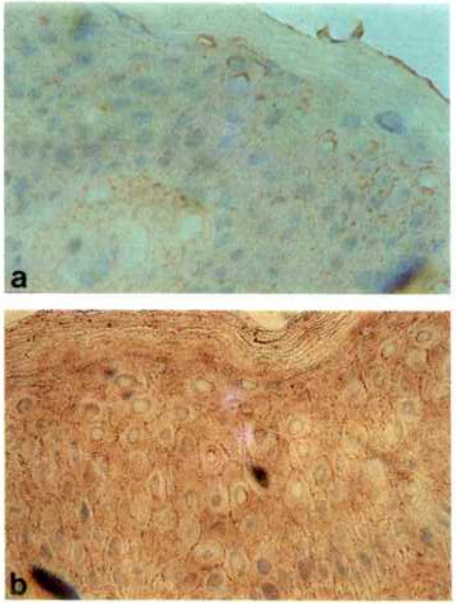

FIGURE 2. Epidermal *in vivo*-bound TNF demonstrated with a biotin-avidin-peroxidase technique (original × 800). (a) Unexposed skin from a normal subject. (b) UVB-irradiated skin from the same subject.

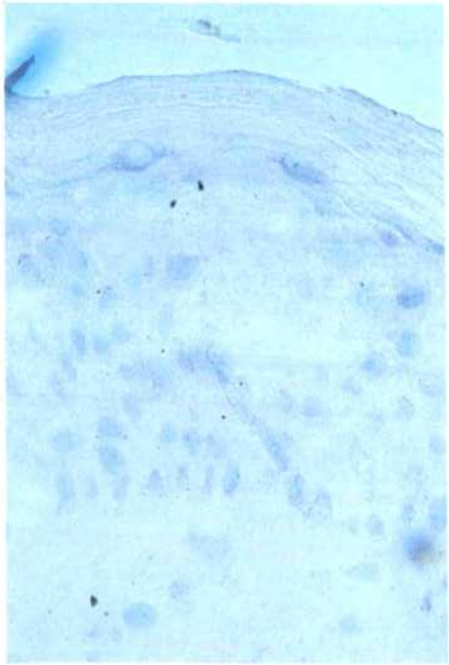

FIGURE 3. Negative control from unexposed skin of a normal subject. The primary layer antibody (anti-cytokine antibody) has been replaced by normal rabbit serum in the biotin-avidin-peroxidase technique (original × 800).

TABLE 1. Maximum dilutions of primary layer antibodies giving epidermal membrane staining using the biotin-avidin technique in skin samples taken before and after irradiation with UVB

	Maximum dilution			
	anti-nIL-1		anti-rTNF	
Subject no.	Non-irradiated	Irradiated	Non-irradiated	Irradiated
1	1:160	1:320	1:160	1:160
2	1:320	1:320	1:320	1:320
3	1:160	1:320	1:40	1:320
4	1:80	1:320	1:20	1:160
5	1:80	1:160	1:40	1:160

cytokines and the fact that skin sections incubated with rabbit sera from nonimmune animals consistently failed to stain with the secondary layer antibody.

The antiserum to human rTNF was monospecific and raised in rabbits immunized with purified human rTNF. The ability of this antiserum to bind to epidermal cells was inhibited only by the specific antigen.

So far TNF has been detected only in the media of BCG/endotoxin-stimulated monocyte-macrophage cultures and mitogen-stimulated blood leukocytes. Activated monocyte-macrophages are therefore considered the major cellular source of TNF.[3,5] Production of TNF by epidermal cells has not been reported previously to our knowledge. Our findings of *in vivo*-produced TNF in the skin and its association with the epidermal cells strongly suggests that this cytokine is produced by keratinocytes and probably also by Langerhans cells.

One can only speculate about the significance of this observation. It is interesting that the quantity of TNF was found to be increased after irradiation with UVB, because UV-irradiation (PUVA) is used successfully in the treatment of skin-lymphomas such as mycosis fungoides.[11]

The anti-nIL-1 antiserum recognizes the three predominant forms of monocyte-derived human IL-1, IL-1α (pI 5), IL-1β (pI 7) and a third component (pI 6 IL-1),[12,13] but it does not bind to other cytokines such as interferon γ, lymphotoxin, TNF or interleukin 2.[10] In normal skin, the antigens visualized by this antiserum seem to be expressed only in areas containing the most mature, and possibly, already stimulated, epidermal cells, as the antibodies failed to bind to the basal cell layers of the epidermis. However, these antigens are probably not completely identical with the α- or β-forms of monocyte-derived IL-1. Thus, we were unable to block the staining by preincubating the antiserum with excess amounts of *E. coli*-derived rIL-1α and rIL-1β. Moreover, a highly purified preparation of monocyte-derived human nIL-1, containing predominantly the IL-1β form, was also ineffective. This may be explained by recent findings that ETAF is only partially identical with the monokine species IL-1α and -β. Thus, Sauder *et al.*[14] showed a homology of less than 40% between human ETAF and IL-1α or -β, and anti-nIL-1 only partially neutralized purified ETAF.[15] We are now investigating whether the IL-1/ETAF antigen is related to the pI 6 form of monocyte IL-1.

ETAF shares many of the immuno-regulatory and stimulatory properties of monocyte IL-1.[1] It may therefore, play a role in proliferative and inflammatory skin processes. IL-1/ETAF augments phytohaemagglutinin-induced thymocyte proliferation, IL-2 production by T lymphocytes and the production of acute-phase proteins by hepatocytes. It also induces neutrophil and monocyte chemotaxis and fibroblast proliferation.[1] It has recently been proposed that UVB may alter the production of IL-1/ETAF by macrophages and epidermal cells *in vitro* and *in vivo*, using animal models.[16–18] In order to study this in man, we used a human *in vivo* model, with a UVB exposure dose based on clinical evaluation. The level of irradiation (3 MED; about 600 mJ/cm^2) and the time of biopsy (24 h after exposure) corresponded to the parameters which Ansel *et al.*[16] found to give a maximum ETAF response.

Ansel *et al.*[16] also showed that sublethal doses of UVB *in vitro* and *in vivo* were capable of increasing production of human and murine ETAF. Gahring *et al.*[17] reported unchanged ETAF activity *in vitro* after short term UVB, despite significant loss of cell viability. Comparative analyses in mice of normal and chronically UVB-exposed skin showed equivalent potentials to secrete ETAF *in vitro* on a per cell basis, as the enhanced production of this cytokine after irradiation with UVB was associated with a significant hyperplasia of the epidermis in the UVB-treated skin.

The results of our *in vivo* studies on human skin support these findings. In normal skin, IL-1/ETAF was related predominantly to the most mature epidermal cells. After irradiation with UVB we found more extensive and intense staining of IL-1/ETAF in the epidermis including the basal cell layer. However, our results do not justify any conclusions concerning the secretion of IL-1/ETAF.

Langerhans cells (LC) reside in the epidermis. It has been shown that UVB can alter the antigen presentation by epidermal cells, probably LC,[18,19] and LC have been shown to secrete IL-1.[1] Nevertheless, LC did not appear to be solely responsible for the expression of IL-1/ETAF staining, because intense staining was seen also in areas without OKT6-positive cells. We did not observe any alterations in LC morphology or location 24 h after UVB.

Immunohistological techniques for the detection of epidermal cytokines may be a valuable supplement to functional tests in the diagnosis of skin diseases. The methods are simple and reproducible and potentially useful in the development and monitoring of local and systemic treatment of skin disorders.

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The prevalence of thyroid autoantibodies in dermatitis herpetiformis

A.P.WEETMAN, J.M.BURRIN, D.MACKAY, J.N.LEONARD*, C.E.M.GRIFFITHS* AND L.FRY*

Department of Medicine, Royal Postgraduate Medical School, London and * Department of Dermatology, St Mary's Hospital, London, U.K.

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SUMMARY

The prevalence of IgG class thyroglobulin and microsomal antibodies, estimated using a sensitive ELISA, was 48% in 115 patients with dermatitis herpetiformis, which was significantly greater than the prevalence of 16% in 107 unselected controls without dermatitis herpetiformis. IgA class thyroid antibodies were found in 29% of dermatitis herpetiformis patients. Overt thyroid disease had been diagnosed in six (5%) of the dermatitis herpetiformis group and a further six patients had elevated TSH levels. The presence of thyroid antibodies was not associated with particular HLA-DR antigens. These results demonstrate the frequent occurrence of thyroid antibodies in dermatitis herpetiformis, although thyroid failure is less commonly associated with this condition. Immune response genes outside the HLA-DR region may be involved in the immune hyper-responsiveness seen in dermatitis herpetiformis which is reflected in the high prevalence of thyroid autoimmunity.

Dermatitis herpetiformis (DH) seems to result from the interaction of generalized immune hyperreactivity, which is probably genetically determined, and gluten sensitivity.[1] The condition is characterized by deposition of granular IgA in the papillary dermis. A feature of the immune hyperreactivity in DH is the occurrence of multiple autoantibodies, including antinuclear factors and antibodies against reticulin, gastric parietal cells and thyroid antigens.[2,3]

However, the exact prevalence of thyroid autoantibodies in DH is not clear. For thyroglobulin (Tg) antibodies detected by haemagglutination, reports range from 0%[3,4] to a prevalence of 14%,[2] while the prevalence of microsomal (M) antibodies ranges from 17–21%[2–4] to 38%.[5] The latter authors also reported a high incidence of clinical thyroid disorders in their patients, including symptomatic goitres and thyroid nodules, for which there was no obvious explanation. Others have suggested an association of thyrotoxicosis with DH, on the basis of uncontrolled case reports.[6]

Correspondence: Dr A.P.Weetman, Department of Medicine, Level 5, Addenbrooke's Hospital, Cambridge CB2 2QQ, U.K.

To determine how commonly thyroid autoimmunity and dysfunction occurs in DH, we tested 115 patients, using specific and sensitive ELISAs for Tg and M antibodies and for thyroid stimulating hormone (TSH). We also investigated whether any characteristic pattern of thyroid antibody change occurred over a period of 5 years and whether there was any HLA-DR association with thyroid autoimmunity in DH.

METHODS

Patients

Fifty women (mean age 49 years, range 12–82 years) and 65 men (mean age 55 years, range 19–84 years) with DH were studied without any prior selection. Sera were stored at −20°C. Sera were also obtained from 42 women (mean age 43 years, range 18–64 years) and 65 men (mean age 49 years, range 20–62 years) without DH, who were hospital personnel, irrespective of family or personal history of thyroid autoimmune disease, to determine the prevalence of thyroid autoantibodies in a control group.

ELISA for thyroid antibodies

Assays for IgG class Tg and M antibodies were performed as described elsewhere using a 1:100 dilution of serum.[7] Sera were regarded as positive for Tg or M antibodies if the absorbance was > 3 SD above the mean for 20 normal (antibody negative) sera included in each assay. We also tested 69 patients for IgA class Tg and M antibodies, in view of the relatively common occurrence of these antibodies in Graves' disease and Hashimoto's thyroiditis[8] and the probable importance of IgA in the pathogenesis of DH.[1] The method used for detection of IgA class antibodies was a modification of the basic ELISA and is described in detail elsewhere.[8]

Assay for thyroid stimulating hormone

Serum TSH was measured using an enzyme-amplified immunometric assay (IQ Bio, Cambridge, U.K.). The reference range in our laboratory is 0·3–3·8 mU/l and the detection limit 0·03 mU/l.

Tissue typing

Seventy seven patients, chosen at random, were tissue-typed using standard methods as described previously.[9]

RESULTS

Prevalence of thyroid antibodies

The prevalence of thyroid autoantibodies is shown in Table 1. The overall prevalence of IgG class thyroid antibodies (either Tg or M, or both) was 48%, compared with 16% in the controls ($P<0{\cdot}001$; χ^2 test). There were fewer subjects with IgA class antibodies in both groups, but again with a significant excess in the DH group ($P<0{\cdot}002$; χ^2 test). IgA antibodies in the absence of IgG antibodies were found in three DH patients and two control subjects; 40% of the IgG antibody positive DH patients had IgA thyroid antibodies.

The sex ratio in the DH patients positive for thyroid antibodies was approximately 1:1 (54% women, 46% men), whereas in the controls the ratio about 3:1 (77% women, 23% men). The mean age in the antibody positive DH group was 50 years (range 26–78 years) and in the antibody negative DH group 53 years (range 12–84 years). This difference was not statistically significant ($P<0{\cdot}1$; Student's t-test).

TABLE 1. Prevalence of thyroid antibodies in DH patients and controls

		No. of subjects with antibodies (%)		
		Tg only	M only	Tg and M
IgG antibodies	DH patients ($n = 115$)*	11 (10%)	10 (9%)	34 (30%)
	Controls ($n = 107$)	4 (4%)	2 (2%)	11 (10%)
IgA antibodies	DH patients ($n = 69$)*	12 (17%)	5 (7%)	3 (4%)
	Controls ($n = 107$)	2 (2%)	0	7 (7%)

* In DH patients where more than one serum sample was available for testing, the result for the most recent sample was used.

Fluctuation in thyroid antibodies

There were varying degrees of change in antibody levels in the 81 DH patients from whom sequential serum samples were obtained, but no consistent pattern was apparent (Fig. 1). Mean M and Tg antibody levels in the first and the last samples were not significantly different ($P > 0\cdot1$; paired t-test). However, in 16 patients (20%), antibodies appeared or disappeared during the 5-year follow-up. Four of these patients had transient M antibodies, one had transient Tg antibodies, three had transient M and Tg antibodies, six developed Tg antibodies and two developed M antibodies. None of the changes could be related to alteration in drug or dietary treatment. Four patients who had transient thyroid antibodies which were not detected in the final sample were not recorded as antibody positive in Table 1; in the other four patients with transient Tg or M antibodies, the other antibody (M or Tg) was not transient and the patient was therefore included in Table 1 as antibody positive.

TSH measurements

Six patients (three men, three women) were known to have thyroid failure, two (one man and one woman) due to iatrogenic hypothyroidism following treatment for Graves' disease. These patients were taking thyroxine at the time of analysis (performed on the most recent serum sample). A further six patients had an elevated TSH level (range 3·9–7·1 mU/l). Thus, 12 (10%) of the DH patients had clinical or subclinical hypothyroidism. Eleven of these patients were thyroid antibody positive and this association of hypothyroidism with thyroid antibodies was statistically significant ($P < 0\cdot01$; χ^2 test). There was no significant difference in antibody levels between the patients with thyroid failure and those without ($P > 0\cdot1$; Wilcoxon rank test). Two patients had undetectable TSH levels; one was on thyroxine replacement and the other had no clinical features of hyperthyroidism, was thyroid antibody negative and euthyroid (having normal free T4 and total T3 levels).

Association with HLA-DR

The prevalence of thyroid antibodies in the DH patients according to HLA-DR type is shown in Table 2. There was no significant association with any particular tissue type. In particular, there was no association with HLA-DR3; six of 10 HLA-DR3 negative patients were thyroid antibody positive compared with 30 of 67 HLA-DR3 positive patients ($P > 0\cdot1$; χ^2 test).

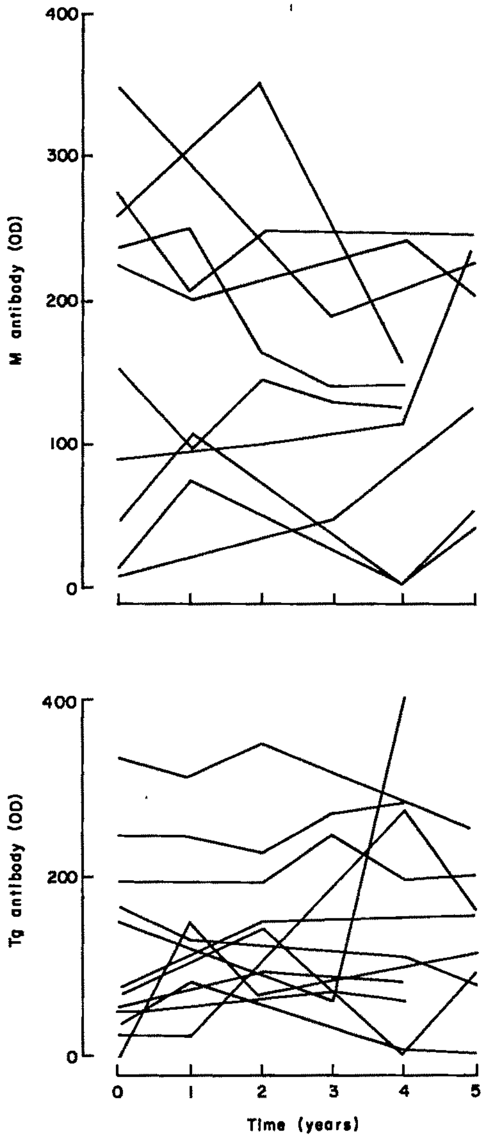

FIGURE 1. Fluctuation in microsomal (M) and thyroglobulin (Tg) antibodies in DH. Results of 12 representative patients are shown (two negative for M antibodies). Antibody levels are shown as absorbance (OD) × 10^{-3} after subtraction of background.

DISCUSSION

This study has shown a significantly increased prevalence of thyroid antibodies in DH. In contrast to other studies[3,4] Tg antibodies occurred with the same frequency as M antibodies. The increased prevalence of thyroid antibodies reported here compared with previous studies[2-4] is probably due in part to the increased sensitivity of the ELISA used to detect thyroid autoantibodies. This would account for the low prevalence (2–4%) of thyroid

TABLE 2. Frequency of HLA-DR phenotypes in DH patients with and without thyroid antibodies

	Percentage of patients	
Antigen	Thyroid antibodies present ($n=36$)	Thyroid antibodies absent ($n=41$)
DR1	11	2
DR2	33	37
DR3	83	90
DR4	22	17
DR5 (DRw11)	8	0
DRw6	3	10
DR7	17	20
DRw8	0	5
DRw9	6	0
DRw10	0	0
DRw12	0	5
DRw13	0	5

antibodies in control subjects in these earlier studies. However, in a more recent study, M antibodies were detected in 38% of DH patients and 12% of controls by haemagglutination;[5] we obtained identical results in the present study using a different assay. The addition of Tg antibody estimations in our patients brings the prevalence of thyroid antibodies in DH to 48%.

It was also noteworthy that the expected increased prevalence of thyroid antibodies in women[9] was not apparent in our DH patients, suggesting that the mechanisms responsible for the increased frequency of thyroid autoimmunity in DH are powerful enough to over-ride the protective effect of male sex. We found a 29% prevalence of IgA class thyroid antibodies, with 40% of IgG class antibody positive patients also having IgA class antibodies. This is slightly less than the 46% prevalence of IgA antibodies we recently found in IgG antibody-positive Hashimoto's thyroiditis,[8] and suggests that there is no disproportionate anti-thyroid IgA response in DH, despite the importance of this class of antibody in the pathogenesis of DH.

Despite the high incidence of thyroid antibodies, clinical and subclinical hypothyroidism were relatively infrequent. Established primary hypothyroidism occurred in 3·5% of our DH patients (two men, two women), while a community survey has shown a prevalence of 0·8–1·1% in the general population, the majority of these patients being women.[9] In the same survey elevated TSH levels were found in 5% of the remaining subjects, which matches that found in our DH patients (5·2%). Thus, there appears to be a slight (up to three fold) increase in overt hypothyroidism in DH, particularly in men, but the prevalence of subclinical hypothyroidism seems the same as in the rest of the population. We could find no evidence for an association of DH with thyrotoxicosis, as has been suggested previously.[6] There were two cases (1·7%) of established hyperthyroidism in the DH patients compared to an expected prevalence of 1·1–1·6% in the general population.[9]

Hashimoto's thyroiditis is associated with HLA-DR4 or HLA-DR5 whereas atrophic thyroiditis is associated with HLA-DR3.[10–13] There have been no reports to our knowledge of

HLA-DR associations in subclinical hypothyroidism with thyroid autoantibodies. In our DH patients there was no significant association of any HLA-DR type with the presence of thyroid autoantibodies. There is a strong association of HLA-DR3 with DH[9,14] and it has been suggested that this common immunogenetic predisposition may account for the association of thyroid disease with DH.[15] Our results argue against such a simple explanation.

However, the reasons for the association of HLA-DR3 with DH are unclear. The association of HLA-DR3 with coeliac disease,[9] in which there is no apparent increase in thyroid autoimmunity,[16] argues against an effect of HLA-DR3, splenic atrophy, or a shared immune response to gluten. However, heterogeneity within the DR3 phenotype, not detectable by conventional serology, could explain this difference, or there could be an effect of non-DR-encoded immune response genes. More refined analysis with HLA-D region probes should allow investigation of this. Also, there is an increased frequency of HLA-DR2 in DH and HLA-DR7 in coeliac disease, which may contribute to the differences between these two conditions.[9]

Patients with DH have polyclonal activation of circulating B cells, but even within the HLA-B8-DR3 haplotype, patients are heterogeneous[17] again suggesting a non-DR3-encoded immune response gene. Such polyclonal activation could lead to the production of multiple autoantibodies, including those directed against the thyroid. The marked fluctuation in thyroid antibodies demonstrated in the present study, and their appearance or disappearance in 20% of the DH patients is different to the infrequent changes which occur in asymptomatic subjects drawn from population surveys[18,19] and would be compatible with fluctuations in the intensity of some unknown polyclonal activator in DH. Polyclonal activation of B cells in DH, (in the absence of activation of cellular immune mechanisms), would also explain the frequent occurrence of other autoantibodies in this condition without the concurrent appearance of clinical disease.[4]

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Comparison of different methods for enumeration of Langerhans cells in vertical cryosections of human skin

T.BIEBER, J.RING, AND O.BRAUN-FALCO

Department of Dermatology, Ludwig-Maximilian University, Munich, West Germany

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SUMMARY

We have quantified Langerhans cells (LC) in cryosections of normal human skin and lesional skin from patients with atopic eczema and psoriasis vulgaris using six different methods. The results from the different methods varied considerably and were sometimes contradictory, for example when LC numbers in psoriatic skin were compared with those in normal skin. Thus, in addition to the staining technique used and the selection of the dendritic cell type to be counted, the enumeration method used can also influence the quantitation of LC in normal and pathological skin.

Since the early work of Wolff and Winkelmann,[1] Silberberg[2] pointed out a role of Langerhans cells (LC) in allergic contact dermatitis, and much interest has been focused on these dendritic cells, considered to be highly specialized antigen presenting cells in the epidermis.[3] Using the ATPase technique, the epidermal LC population has been studied quantitatively in normal skin[1] and in various pathological conditions.[4] This technique, using fresh epidermal sheets, remained the most reliable staining method of LC for light and electron microscopy until it was possible to study cryosections with specific monoclonal antibodies. It is now known that LC have several surface antigens (CD4, T200 and HLA-DR) and cytoplasmic antigens (S-100) some of these in common with other cell types. One of the most specific markers of LC seems to be CD1 (T6)[5] which is only shared by the so called indeterminate cells (IC)[6], considered to be LC precursors, and thymocytes[5] which are not usually found in normal or pathological epidermis. Therefore, numerous investigations using in situ immunophenotyping of LC in different skin diseases have been published. These studies mostly demonstrated changes in the LC population with respect to the distribution in the epidermal compartment. The interpretation of such studies is often difficult since the methods for enumeration of LC—especially when using vertical sections—vary considerably from one investigator to another.

Correspondence: Dr Thomas Bieber, Department of Dermatology, University of Munich, Frauenlobstrasse 9-11, D-8000 Munich 2, West Germany.

Therefore, we have compared six different methods for quantitative assessment of LC in vertical cryosections using the monoclonal antibody OKT6 (anti-CD1) on skin biopsies from normal and acanthotic epidermis (lesional skin from chronic atopic eczema and chronic psoriasis vulgaris).

METHODS

Subjects and biopsy specimens

Punch biopsies (6 mm) were taken under local anaesthesia from the back skin of five healthy volunteers and from the same area of involved skin of five patients with chronic lichenified atopic eczema and five patients with chronic plaque type psoriasis. The lesions had been untreated for at least 4 weeks prior to biopsy. The biopsy specimens were divided into two parts; one was fixed in 4% formaldehyde for routine histology and the other was immediately frozen for immunohistochemistry.

Immunohistochemistry

Reagents. Orthoclone OKT6 monoclonal antibody (Ortho Immunobiology Ltd, Raritan, NJ U.S.A.) has previous been shown to react with 70% of human intrathymic lymphocytes and specifically with epidermal LC and IC.[5,6]

Labelling procedure. Immunolabeling was performed using a modified alkaline-phosphatase mouse anti-alkaline-phosphatase complex (APAAP) method as described by Cordell *et al.*[7] All incubations were carried out at room temperature in a moist chamber. Frozen sections (6 μm), after drying at room temperature, were fixed in pure cold acetone (10 min at 4°C), and incubated with OKT6 (final dilution 1:100) for 30 min. The sections were rinsed twice with Tris buffered saline (TBS) (1:9 Tris-HCl 0·5 M, pH 7·6 and 0·15 M saline) for 10 min and incubated for 30 min with a sheep anti-mouse antibody (Dako 2259) diluted 1:20 in RPMI 1640 containing 5% inactivated human serum. After washing twice for 10 min in TBS, the sections were incubated for 30 min with the APAAP complex (Dako D651), final dilution 1:25 in RPMI 1640. Then the sections were rinsed twice in TBS for 10 min and then incubated for 15 min with the substrate N-AS-BI-phosphate (Sigma) (125 mg N-AS-Bl phosphate dissolved in 1·5 ml dimethylformamide and then in 250 ml distilled water with 62·5 ml 0·2 M TBS and with 90 mg levamisole to block endogenous alkaline phosphatase activity). Immediately before staining, 50 mg sodium nitrite and 0·5 ml astral fuchsin were dissolved in the substrate solution and filtered directly onto the slides.

Enumeration methods. Three sections of each skin biopsy were examined and only dendritic cells exhibiting a brightly stained cytoplasm with a dark nucleus were counted (Figs 1 and 2). LC density in the epidermis was then calculated by six different methods: (1) LC/20 high power fields (× 40 objective). (2) LC/mm length of epidermal surface, measured by morphometry. (3) LC/mm length of basement membrane, measured by morphometry. (4) LC overlying 200 basal cells. (5) LC/mm^2 epidermal section surface counted with an ocular square grid covering 0·03062 mm^2 with a × 40 objective. (6) LC/mm^2 epidermal section surface measured by morphometry. For this method, the surface of each defined section was measured by computer assisted morphometry (KONTRON) in adjacent areas. The LC were counted in the same area using a × 40 objective.

For each area considered, the ratio of the length of basement membrane zone underlying the

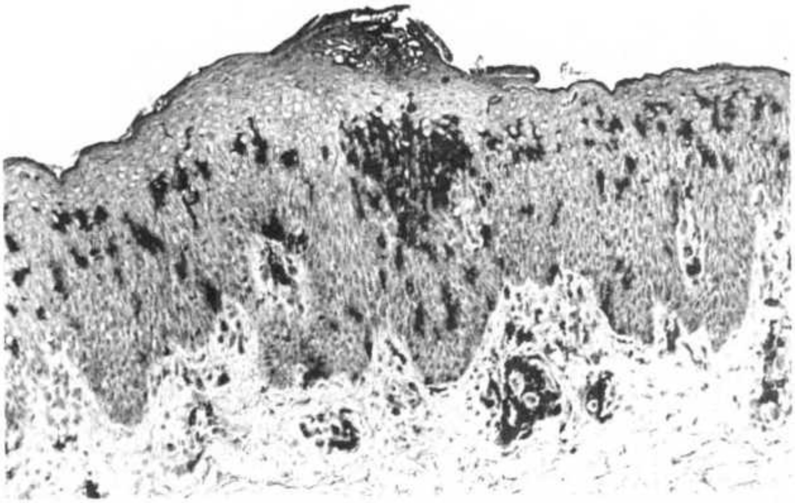

FIGURE 1. Lesional skin from atopic eczema. CD1 (OKT6) positive cells stained with the APAAP method. (Original × 100).

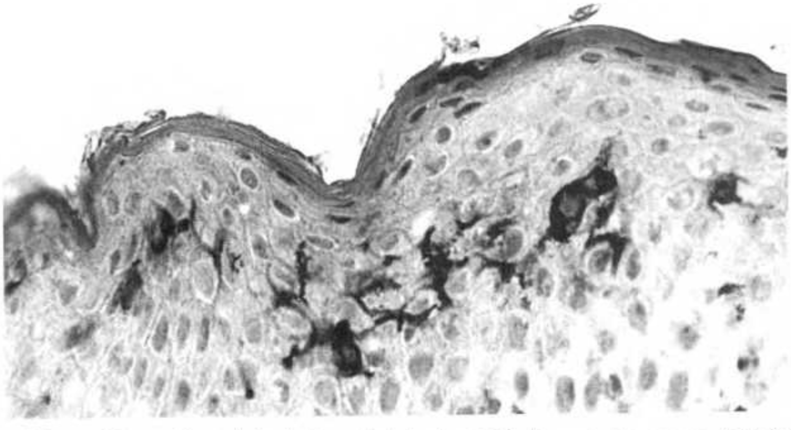

FIGURE 2. CD1 positive epidermal dendritic cells in lesional skin from atopic eczema. (Original × 400).

length of the stratum corneum was calculated to evaluate papillomatosis. The length of basement membrane zone beneath 200 basal cells was also calculated.

Statistical analysis

The results were analysed using Student's t-test.

TABLE 1. Enumeration of epidermal Langerhans cells (LC) using unidimensional measurement methods

Method of measurement	Number of LC (mean ± SD) Normal skin	Psoriatic skin	Atopic eczema
(1) LC/20 high power fields	96 ± 47	105 ± 35	150 ± 56*
(2) LC/mm length of epidermal surface	9·08 ± 3·15	9·72 ± 5·20	24·66 ± 7·11***
(3) LC/mm length of basement membrane	5·78 ± 2·05	3·30 ± 1·20†	10·73 ± 2·30***
(4) LC/200 basal cells	8·85 ± 2·29	5·14 ± 2·28‡	14·57 ± 3·07**

Significantly greater than in normal skin *$P < 0{\cdot}02$, **$P < 0{\cdot}005$, ***$P < 0{\cdot}002$.
Significantly less than in normal skin †$P < 0{\cdot}05$, ‡$P < 0{\cdot}02$.

TABLE 2. Enumeration of epidermal Langerhans cells (LC) using two-dimensional methods

Method of measurement	Number of LC (mean ± SD) Normal skin	Psoriatic skin	Atopic eczema
(5) LC/mm^2 grid	73·6 ± 36·4	103·0 ± 35·1	196·6 ± 35·3*
(6) LC/mm^2 morphometry	101·2 ± 34·2	46·6 ± 25·6†	117·2 ± 42·7

* Significantly greater than in normal skin, $P < 0{\cdot}02$.
† Significantly less than in normal skin, $P < 0{\cdot}05$

RESULTS

There were marked differences in LC counts according to the method used. The results are presented in Tables 1 and 2.

(1) *LC/20 high power fields*

There was no significant difference between the mean LC count in normal and in psoriatic skin, but in atopic eczema the LC count was significantly higher than in normal skin ($P < 0{\cdot}02$).

(2) *LC/mm length of epidermal surface*

With this method we found a statistically significant difference between LC numbers in normal skin and atopic eczema ($P < 0{\cdot}002$), but no significant difference between psoriatic and normal skin.

(3) *LC/mm length of basement membrane*

The numbers of LC were significantly greater in atopic eczema than in normal skin ($P < 0{\cdot}002$) and significantly lower in psoriatic skin than in normal skin ($P < 0{\cdot}05$).

(4) *LC/200 basal cells*

Similar results were obtained to those found with method 3. Numbers of LC were significantly higher in atopic eczema than in normal skin ($P < 0.005$) and significantly lower in psoriatic skin than in normal skin ($P < 0.02$).

(5) *LC/mm² epidermal section surface, measured by ocular grid*

The counts with this method were very different from those obtained with the other methods. There were significantly more LC in atopic eczema than in normal skin ($P < 0.02$). Numbers of LC in psoriatic skin were also higher than in normal skin, but this difference was not statistically significant.

(6) *LC/mm² epidermal section surface measured by morphometric analysis*

This method showed no statistically significant difference between LC numbers in atopic eczema and in normal skin, but significantly lower numbers in psoriatic than in normal skin ($P < 0.05$). The degree of papillomatosis evaluated by the ratio mm length of the basement membrane zone/mm length of epidermal surface in the same area, gave the following results: normal skin = 1·56, atopic eczema = 2·29 psoriasis vulgaris = 2·97.

DISCUSSION

Since it has been established that OKT6 and other anti-CD1 monoclonal antibodies (e.g. BL6, NA1/34 and Leu-6) specifically recognize LC or IC[5,6] many immunohistochemical studies have described variations in the LC population in various skin diseases. However, in most of these investigations different enumeration methods were used which makes the results rarely comparable and sometimes contradictory. Therefore, we considered it of interest to compare some of the most widely used enumeration techniques in order to understand better the discrepancies between results obtained in particular diseases.

In the present study, using six different enumeration methods on the same sections, we have shown an impressive degree of variability in the results depending on the method used and the type of disease investigated.

In order to standardize these methods, firstly the characteristics of the cells investigated must be defined. Recently, de Jong *et al.*[8] described three types of dendritic patterns of epidermal LC depending upon the section thickness and recommended distinguishing these types in the enumeration. However, although different patterns may reflect various functional states of LC, for practical purposes in the present study we did not differentiate between different types of LC. Considering each section not as a surface, but as a part of a volume where LC nuclei are visible at the cut surface, the thickness of the sections represents an important parameter for calculating the real density of LC per section. Thus, a slight change in the section thickness would produce an important change in the cell count.

Enumeration of LC by counting a defined number of high power microscopic fields[9] is one of the most convenient methods, because no special equipment is needed. This method is suitable for acanthotic epidermis where a large part of the microscope field is occupied by epidermis, or for normal skin if only uninvolved or normal skin is being studied. As soon as normal skin was to be compared with pathologically altered involved skin, we found this method unsuitable because it does not differentiate between pathological epidermis (with acanthosis, papillomatosis or atrophy) and normal epidermis. In acanthotic and papillomatotic conditions, an increase in the LC population is normally found, although by other methods (see Tables 1 and 2) the LC population is found to be significantly decreased.

Therefore, several authors have recommended the enumeration of LC per mm length of epidermal surface[10-12] as a simple and reproducible method for cryosections or paraffin sections.[13] This is based on the consideration that the number of LC should be independent of epidermal thickness. In our study, the two types of acanthotic epidermis examined gave different numbers of LC when evaluated by this technique. There was no difference between psoriatic and normal skin, whereas in atopic eczema LC numbers were markedly increased. The LC numbers in normal skin were comparable—relative to the section thickness—to previously published values.[14-17]

Quantitation of LC by enumeration per mm length of basement membrane zone[13,18] can be considered as a simple and convenient method for normal and acanthotic skin. However, in contrast to enumeration per mm length of epidermal surface, because of the convoluted basement membrane zone, a morphometric analysis system is required for the exact measurement of length. With this quantification technique, the epidermal thickness is reflected more reliably. With this method we found a significant decrease in LC in psoriasis vulgaris but an increase in atopic eczema compared with normal skin. This shows that the density of the LC pool is not identical in different diseases with acanthosis and papillomatosis.

Another possibility for quantification considering the basement membrane zone is counting LC per 200 basal cells.[19,20] In our hands the results with this method were in accordance with the enumeration per mm length of the basement membrane zone. However, when we measured the actual length of basement membrane zone underlying 200 basal cells there was no constant relationship. In normal and psoriatic skin, 200 basal cells corresponded to a basement membrane length of 1·54 mm and 1·57 mm, respectively whereas in atopic eczema 200 basal cells corresponded to only 1·35 mm. Thus, in view of these variations, there is evidence that these two methods do not exactly correlate, in contrast to previous reports.[21] Some authors have found significant differences between normal and psoriatic skin by measuring LC/mm epidermal surface and LC/200 basal cells.[19] Additional problems will arise in counting basal cells if apart from acanthosis there is papillomatosis and the epidermal area includes dermal papillae. Moreover, in some diseases with partial destruction of the stratum basale through hydropic degeneration (e.g. in lichen planus), an enumeration per 200 basal cells may be impossible.

In contrast to the above methods which are all related to a unidimensional parameter, the quantification of LCs per mm^2 epidermal section surface uses a two-dimensional scale. We performed this type of LC assessment in two different ways, using an ocular square grid[22,23] and by morphometric analysis (Bieber and Braun-Falco, unpublished) and found contradictory results. When the section surface is evaluated with an ocular square grid, the investigator has difficulty counting the epidermal section precisely and tends to over- or underestimate. The impossibility of adapting the grid to a convoluted configuration in papillomatosis may play a role, as well as the tendency to select areas with positive staining.

Morphometric analysis allows an exact evaluation of the area under investigation. We have reported previously that with this method no significant differences could be detected between normal skin and atopic eczema[24] where a subpopulation of dendritic cells bearing IgE receptors may be of particular interest in the pathogenesis of this disease.[25] The marked decrease in LC in psoriasis could be explained by a possible migration from the epidermal compartment into the dermal infiltrate.[26]

In the present study, we have enumerated LC on vertical cryosections using six different methods, in normal skin and in atopic eczema and psoriasis vulgaris. The results obtained differed substantially from one method to another, particularly the differences between psoriatic and normal skin. The LC count in normal skin (enumerated by any method) is known to vary widely from subject to subject,[18] from site to site, and possibly on different occasions, so that the determination of a normal count, especially when obtained from a small number of subjects as in the present study, should be regarded with caution. Like other authors,[27] we feel that the major problem in the choice of an appropriate assessment method is linked to the understanding of the role of the LC in the epidermis. Thus, if one accepts that LC are only supposed to form a suprabasal network, a unidimensional parameter (for example LC/mm length of epidermal surface) can be used. Quantification of LC per 200 basal cells seems to be ideal on the basis of the theory of an epidermal proliferation unit. However, with regard to the concept that LC act as antigen presenting cells for the whole epidermal volume, enumeration in a two-dimensional area of the epidermis using a morphometric analysis system seems to be an appropriate method.

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Genetic control of sebum excretion and acne—a twin study

SHERNAZ WALTON†*, E.H.WYATT† AND W.J.CUNLIFFE*

†Department of Dermatology, Hull Royal Infirmary, Hull, and *Department of Dermatology, The General Infirmary, Leeds, U.K.

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SUMMARY

Sebum excretion and acne grades were measured in 20 pairs each of identical and non-identical like-sex twins. The identical twins had virtually identical rates of sebum excretion ($P > 0{\cdot}05$), but they had a significantly different degree of acne severity ($P < 0{\cdot}01$). The non-identical twins had significantly different sebum excretion rates ($P < 0{\cdot}01$) and acne grades ($P < 0{\cdot}01$). These findings suggest that sebum excretion is under genetic control and the development of clinical lesions is modified by environmental factors.

Acne vulgaris is probably an inherited disease; although the precise mode of transmission is not known it is probably multifactorial. In one study, 82% of patients with acne gave a history of acne in at least one sibling and in 60%, a history of acne was obtained from one or both parents.[1] A twin study has shown that there is almost 100% concordance for occurrence of acne in identical twins. To our knowledge there has been no study of the severity of acne or the sebum excretion rate in twins.[2]

It is not known which aetiological factors for acne are inherited. It has been suggested that acne vulgaris represents an exaggerated response of the pilosebaceous unit to normal levels of circulating androgens. An alternative explanation includes 'genetically' mediated elevation of circulating androgens.[3,4] Ways of investigating the genetic mediation of acne are many and the investigation of twins is a useful biological system. We have assessed acne grades and measured sebum excretion rate in identical and non-identical like-sex twins.

METHODS

Patients

Twenty pairs of identical twins (five male and 15 female) aged between 14 and 26 years (median 15 years) and 20 pairs of non-identical twins of the same sex, (12 male and eight female) aged

Correspondence: Dr S. Walton, Department of Dermatology, Hull Royal Infirmary, Anlaby Road, Hull, N. Humberside, HU3 2JZ, U.K.

between 12 and 18 years (median 15 years) were included in the study. Informed consent was obtained from all subjects whose names were obtained from a Hull-based register.

Method

The acne was graded by the technique of Burke and Cunliffe[5] and the sebum excretion rate (SER) was measured on the forehead by the direct gravimetric technique.[6]

TABLE 1. Severity of acne and sebum excretion rates (SER) in the twins studied. Values are medians and ranges

Twins	Sex	n*	Acne grade	SER $\mu g/cm^2/min$
Identical	Male	10	0·25 (0–2·75)	0·77 (0·07–1·16)
Identical	Female	30	0·20 (0–1·45)	0·52 (0·14–1·28)
Non-identical	Male	24	0·20 (0–0·75)	0·66 (0·18–1·29)
Non-identical	Female	16	0·20 (0–1·00)	0·50 (0·04–1·45)

* Number of individuals

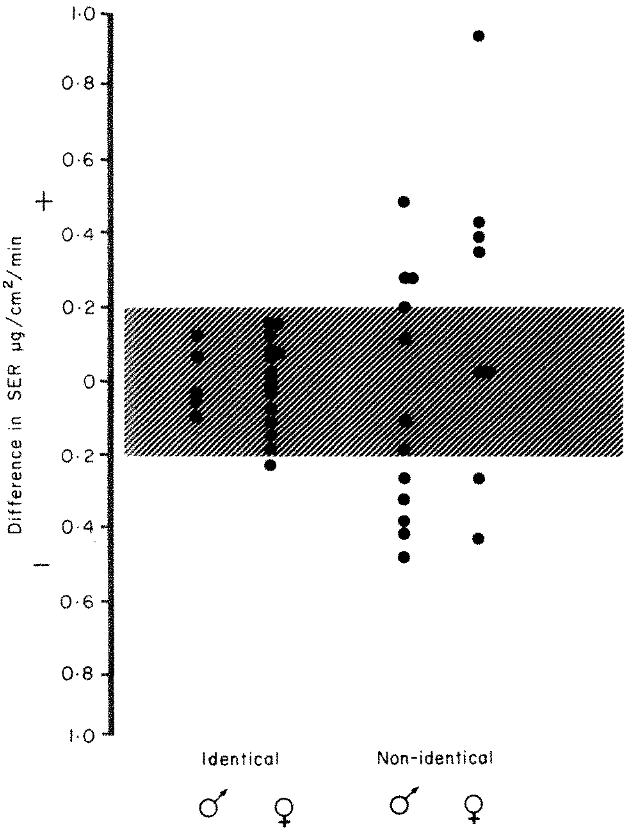

FIGURE 1. Differences between sebum excretion rates in identical twins and in non-identical twins.

Statistical analysis

The results were expressed as differences between individual pairs of twins analysed by analysis of variance.

RESULTS

Median acne severity and sebum excretion rate in identical and non-identical twins of each sex are shown in Table 1. Differences in SER and in acne grade between the twins in each pair are shown in Figures 1 and 2 respectively. The identical twins had similar sebum excretion rates ($P > 0{\cdot}05$), but they had significantly different grades of acne severity ($P < 0{\cdot}01$). The non-identical twins differed significantly in sebum excretion rate ($P < 0{\cdot}01$) and acne grade ($P < 0{\cdot}01$). Only one pair out of 20 identical twins had a difference in SER outside the range $0 \pm 0{\cdot}2$ $\mu g/cm^2/min$, whereas fourteen out of 20 pairs of non-identical twins had a difference in SER outside this range. On the other hand, there was a similar scatter of differences in acne grades in identical and non-identical twins.

The incidence of acne in both groups was similar; 16 out of 20 pairs in each group had evidence of acne.

DISCUSSION

The importance of genetic factors in determining susceptibility to acne is suggested by genetic[7] and racial[8] studies and is confirmed by the very high degree of concordance between identical

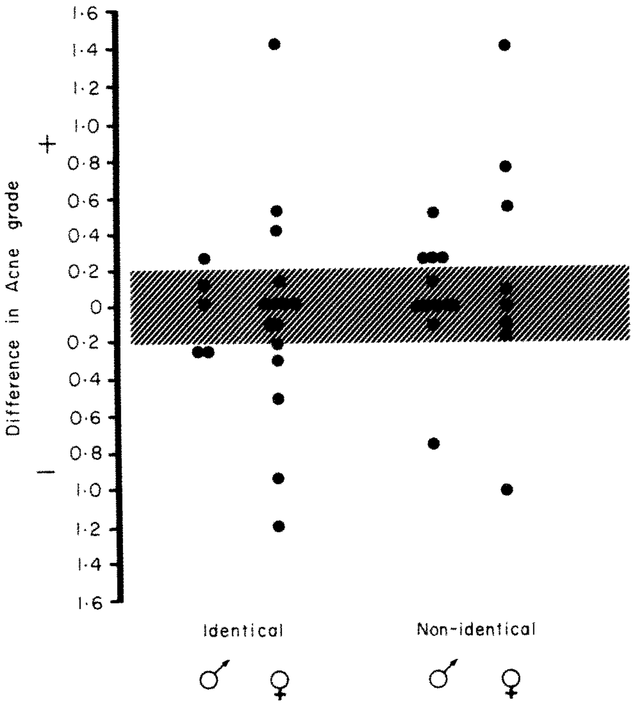

FIGURE 2. Differences between acne grades in identical twins and in non-identical twins.

twins[2]. A recent twin study on the composition of sebum, showed negligible intra-pair differences in the proportions of *iso*-even fatty acids in sebaceous wax esters in identical twins.[9] This lends further support to a possible genetic component in the control of sebum production. A survey in Germany showed that acne had been present in one or both parents of 45% of schoolboys with acne, but in parents of only 8% of boys without acne.[10]

The mode of inheritance of acne is unknown, but is assumed to be multifactorial. Four major aetiological factors for acne have been identified: increased sebum production, an abnormality of the microbial flora, hypercornification of the pilosebaceous duct and the production of inflammation. Whatever the relative importance of the various aetiological factors may be, our finding of almost identical sebum excretion rates in identical twins and dissimilar rates in non-identical twins supports the idea that sebum excretion is under genetic control. However, the dissimilar grades of acne severity in identical and non-identical twins suggests that the development of acne lesions is modified by environmental factors. Since our acne grading system gives a score which reflects more the degree of inflammation than comedonal status, we suggest that the most likely environmental factors inducing acne lesions are the growth rate of *Propionibacterium acnes* and its production of biological mediators of inflammation. The skin surface and ductal bacteria are in reality external to the body—their growth and function being modified by the skin micro-environment.[11]

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Tissue measurements in senile sebaceous gland hyperplasia

P.KUMAR, S.P.BARTON AND R.MARKS

Department of Medicine (Dermatology), University of Wales College of Medicine, Heath Park,
Cardiff CF4 4XN, U.K.

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SUMMARY

Senile sebaceous gland hyperplasia has been studied in 18 subjects in an attempt to shed some light on its pathogenesis. The sizes of different segments of the pilosebaceous unit were compared in senile sebaceous gland hyperplasia (SGH) and in control skin samples, using stereological techniques. The whole follicle was found to be significantly increased in volume compared with follicular structures in the control specimens. The size of differentiated sebaceous gland cells was found to be reduced compared with controls (490 ± 72 μm^2 and 554 ± 98 μm^2, respectively), but not significantly so. The mean tritiated thymidine autoradiographic labelling index determined *in vitro* was found to be decreased ($5{\cdot}42 \pm 1{\cdot}79$) compared with controls ($7{\cdot}48 \pm 2{\cdot}44$), but again, not significantly so. In addition the degree of solar elastotic degenerative change was found to be similar in SGH bearing skin compared with control samples. It is of interest that SGH and prostatic hypertrophy are exceptions to the usual atrophy of ageing and that both tissues are androgen dependent.

Sebaceous gland hyperplasia (SGH) is a relatively common disorder usually affecting the elderly, although younger individuals may occasionally also be affected. Despite the fact that original mention of this disorder was made by Unna[1] in 1874 and that in the ensuing years various other workers[2–6] have described the morphology, histology and enzyme histochemistry, the aetiopathogenesis of SGH remains obscure. It is of particular interest with regard to the skin ageing process, as it remains one of the few 'hypertrophic' processes to occur in the face of generalized atrophy of the skin during senescence.

In a previous study we reported that, contrary to popular belief, there did not appear to be an association between SGH and solar elastotic degenerative change.[7] The primary aim of the present study was to determine whether hypertrophic changes affected other follicular structures. It was also hoped to check our previous clinical findings to establish whether solar

Correspondence: Professor R.Marks, Department of Medicine, University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, U.K.

elastotic degeneration had any role to play in the causation of this disorder, using histological techniques.

To obtain further information on the pathophysiology of the disorder we have also studied a measure of the rate of cell production and sebaceous gland cell size in the hypertrophied sebaceous glands.

METHODS

Eighteen patients (12 males and six females) who had clinically evident SGH lesions on the face, and who had given their informed consent, participated in the study. Most of these individuals were found to have SGH lesions on routine clinical examination, but six patients were identified from the dermatopathology records of the dermatology department and were recalled. These six patients had previously had one or two SGH lesions excised because of a mistaken diagnosis of basal cell carcinoma. Only four of these six had developed further lesions.

The control group was five male and five female patients of the same age group who had attended the dermatology outpatients clinic for routine excision of benign lesions (almost all benign naevi). The perilesional normal skin was used for comparison. Care was taken to include only lesions from the same areas as in the study population. The mean age of the male patients was 60 ± 6·8 years and that of male controls was 62·8 ± 4·9 years. The mean age of the female patients was 57·6 ± 8·4 years and of the female controls was 59·6 ± 12·5 years.

SGH lesions were excised using disposable 4 mm trephines. Half of each biopsy was cut into slices and placed in Eagle's medium containing tritiated thymidine (2·5 μCi/ml). Autoradiographs were prepared after *in vitro* incubation for 4 h. Six slides, each with eight sections were prepared from each specimen. Twenty sections between each slide were discarded. The other half of the biopsy was fixed in 10% formalin and used for histological measurements.

In all individuals a note was also made of any other dermatological or medical illness. The degree of solar elastotic degeneration present clinically was scored on a 10 cm visual analogue scale.

The formalin fixed specimens were prepared routinely for haematoxylin and eosin staining. Eight slides with five sections each were prepared from each specimen. Five sections between each slide were discarded. Similarly a further eight slides were prepared from each specimen and stained with Halmi's stain to assess the dermal structure and the degree of solar elastotic degeneration.

The relative volumes of differentiated sebaceous gland epithelium, non-differentiated sebaceous gland epithelium, sebaceous gland lumen and hair follicle were calculated using stereological point counting procedures.[8] A 10 × 10 lattice, spacing 0·2 mm, was superimposed on the microscopic image and the number of points within the features listed above were counted from at least 28 fields in each patient. For some individuals the number of fields counted was more than 28 in order to complete the particular section. At least four randomly selected slides were counted for each individual.

Autoradiographs were evaluated by counting the number of labelled undifferentiated sebaceous epithelial cell nuclei and the total number of these nuclei, labelled and unlabelled, in randomly selected fields—one from each of at least three lobes—in at least three of the six prepared slides. SGH samples had more lobes and were therefore amply sampled by three fields and three slides. Controls were more exhaustively sampled, but still did not generate the same numbers of nuclei obtained from SGH samples. When counting sections from SGH, care was taken to exclude regions where the tritiated thymidine label had obviously not penetrated. The autoradiographic labelling index (LI) was calculated for each specimen by expressing the number of labelled nuclei as a percentage of the total number of undifferentiated sebaceous cells.

The size of differentiated sebaceous epithelial cells was determined from the sections prepared for stereological point counting. Randomly selected sebaceous gland lobes were viewed, at × 40 objective magnification, concurrently with an LED cursor via a camera lucida. This cursor was attached to a Summagraphic® digitizer tablet, which in turn was interfaced to a HP-85 microcomputer. Software (GIS Ltd) allowed the tracings of the cursor on the digitizer to be converted to calibrated cell area. Cells were systematically selected from the gland cross section by their intersection with a cross-wire incorporated into the eye-piece. This ensured that cells were sampled throughout the sebaceous gland. At least five fields were measured (at least 75 profiles) and mean and median cell area calculated.

Solar elastotic change was determined by assessing at least two sections on randomly selected slides stained with Halmi's stain for elastin. The results were recorded by visual analogue scale (VAS) assessment, with 0 indicating no elastosis and 100 indicating maximal elastosis. This procedure has been found to have a high degree of reproducibility for both intra-observer and inter-observer observations in other types of microscopic evaluation.[9]

Statistical analyses were performed using the non-parametric Mann-Whitney U test. This method was chosen because there are no inherent assumptions about the distribution or equality of variance of the two data sets. This is particularly important for stereological parameters and labelling indices, which are ratios. Since the hypothesis under test predicted the direction of change for gland size, cell size and labelling index[10] a one-tailed test was chosen. The null hypothesis of no difference between SGH and controls was rejected at values of $P < 0.05$.

RESULTS

Of the 18 lesions biopsied, 17 were confirmed as SGH, although one of these was technically unsuitable for inclusion in the study. The remaining specimen was found to be a cellular naevus and was discarded. Two control slides did not shown any sebaceous tissue and were discarded.

The relative volume of the pilosebaceous unit, comprising counts from hair follicle, differentiated sebaceous epithelium, undifferentiated epithelium and pilosebaceous lumen, was significantly greater in patients with SGH than controls (mean 0·377 and 0·187, respectively). Separating out the data from these parts of the pilosebaceous unit showed that this difference was maintained in all components with the single exception of the pilosebaceous lumen. Thus

TABLE 1. Stereological parameters from patients with SGH and controls. All values are volumes (relative to unit volume of dermis) expressed as means ± SD

	Volume relative to dermis		SGH as % of control	P*
	SGH ($n = 16$)	Control ($n = 8$)		
Pilosebaceous unit	0·377 ± 0·125	0·187 ± 0·045	202	< 0·001
Sebaceous gland	0·299 ± 0·116	0·127 ± 0·046	235	< 0·001
Differentiated sebaceous epithelium	0·220 ± 0·091	0·088 ± 0·033	250	$0.001 < P < 0.01$
Sebaceous gland lumen	0·012 ± 0·012	0·010 ± 0·013	120	NS

* Patients and controls compared using a one-tailed Mann-Whitney U test.
NS = not significant.

there was greater hair follicle epithelium in SGH (mean 0·066) than in controls (mean 0·026), more undifferentiated sebaceous epithelium in SGH (mean 0·08) than in controls (mean 0·04), and more differentiated epithelium in SGH (mean 0·220) than in controls (mean 0·088). These data are summarized in Table 1.

The results of measurements of cell production and cell size in the sebaceous gland are summarized in Table 2.

The tritiated thymidine autoradiographic labelling index (LI) in SGH lesions varied from 3·0% to 10·5% (mean 5·92%) and from 4·8% to 10·5% (mean 7·48%) in the control group. This

TABLE 2. Measurement of undifferentiated sebaceous cell production (^3H thymidine autoradiographic labelling index) and the size of differentiated sebaceous epithelium (mean projected sectional area) in patients with SGH and controls. Values are group means ± SD

	SGH ($n=17$)	Controls ($n=6$)	P*
Labelling index (%)	5·92 ± 1·79	7·48 ± 2·24	NS
Mean cell area (μm^2)	490·0 ± 72·0	553·9 ± 97·8	NS

* Patients and controls compared using a two-tailed Mann-Whitney U test.
NS = not significant.

TABLE 3. Visual analogue scale scores for solar elastotic degeneration in patients with SGH and controls

SGH patients		Controls	
No.	Score	No.	Score
1	0·4	1	1·8
2	4·0	2	5·4
3	4·8	3	2·7
4	7·7	4	4·2
5	6·4	5	7·0
6	5·8	6	2·6
7	6·8	7	0·4
8	5·8		
9	6·8		
10	2·8		
11	3·5		
12	5·6		
Mean ± SD	5·03 ± 2·06		3·4 ± 2·24
Median	5·7		2·7

difference was not statistically significant. The mean area of differentiated sebaceous gland epithelium was smaller in SGH lesions (mean 490·0 μm^2) than controls (mean 553·9 μm^2), but this difference was also not statistically significant.

Visual analogue scale scores for solar elastotic degeneration in 12 SGH patients varied between 0·4 and 7·7 and in seven control subjects between 0·4 and 7·0. There was no significant difference between the two groups. The individual values are shown in Table 3.

DISCUSSION

In the study by Luderschmidt and Plewig[10] it was shown that SGH is characterized by large sebaceous glands with small sebocytes. A lower cell turnover and increased numbers of basal cells per unit length gland margin was also suggested, although the ratio of basal cells to sebocytes was not different. It remained unclear, however, whether the hyperplastic process was limited to the sebaceous gland. Our measurements of gland size were accomplished according to stereological principles and are thus not directly comparable. However, the relative volumes of all the tissue compartments suggest that each of these is increased in SGH with the exception of the pilosebaceous lumen. Our study, therefore, shows that the entire pilosebaceous unit undergoes hypertrophy. Our results would agree with the above authors on the ratio of basal cells : sebocytes, which showed no difference between SGH and controls. Our ratio, however, is based on volume and theirs on cell number. This explains the discrepancy between our ratio of 2·75 in SGH and 2·27 in controls, compared to their 1·2 and 1·0, respectively.

The labelling index in SGH was reported by Luderschmidt and Plewig[10] to be decreased from 8·6% to 5·7% using *in vivo* injection of tritiated thymidine. Although our technique was different—*in vitro* incubation of tissue slices with the label—our group means are in general agreement with their values. Our experience, and that of others[11] using both of these methods for labelling basal epidermal cells has not indicated any great discrepancy between *in vivo* and *in vitro* labelling and this would also appear to be the case for sebaceous epithelium, given the similarity of the values. However, our statistical analysis of the results, using a test more appropriate for ratios, did not reveal a significant difference between the groups of data. Luderschmidt and Plewig[10] compared LI from uninvolved sebaceous glands of SGH patients and normal young controls with lesional LI from SGH patients. It is unclear from their report whether the statistical differences were for the (correct) within patient control or whether both young and old controls had been pooled for the analysis.

With respect to individual sebocyte size, our study has shown that this is not different in SGH compared with controls. Again, sampling may be responsible for the disagreement with earlier work. Luderschmidt and Plewig[10] measured projected area of an unstated number of 'centrally located' sebocytes in SGH (471·6 μm^2) and uninvolved glands (941·9 μm^2). The difference in gland size will influence the cells sampled at the 'central location'. Our own measurements were randomly collected and we sampled from all regions of the gland. At least 75 profiles were measured and the 95% confidence limits were always less than 20% of the mean, indicating that this was a representative sample. Our value for SGH—490 μm^2—agrees with the previous work[10] but our control sebocytes, measuring 554 μm^2, were not significantly bigger. The size in the control group also agrees with our measurements from acne patients treated with isotretinoin for 4 weeks (566 μm^2).[12] Further analysis of the present data using median cell area, again showed no statistically significant difference between SGH (490 μm^2) and controls (524 μm^2) thus ruling out a difference in population size distribution.

SGH lesions occur on the forehead and temples in most individuals. Although these areas are commonly believed to be sites of maximal sun damage, in a study by Kligman[13] it was concluded that maximal solar change occurs on the ear and cheek. In our own recent study,[7] 77 elderly individuals with SGH were examined and no correlation between the presence of SGH and the degree of clinical solar damage was found. The present study in which comparisons were made with sections from normal controls, support this conclusion, and it must be assumed that solar degeneration is not a prime cause of SGH.

Graham Smith and Brunot[14] showed that exogenous testosterone resulted in a significant temporary increase in sebum excretion in both aged males and females. It is well known that androgens supply the primary drive to sebaceous gland activity. These hormones cause enlargement of sebaceous glands, increased mitotic activity in sebocytes and increased rates of sebum secretion. It is possible, although unlikely, that an increase in the circulating androgen levels is responsible for SGH. Our initial studies suggest that this is not the case (Marks *et al.* unpublished data).

It is worth noting that most structures show atrophy during the ageing process—the sebaceous glands and the prostate gland being conspicuous exceptions. Interestingly both these exceptions are under androgenic control. SGH is of obvious cosmetic significance to those who suffer from it. It may also have considerable biomedical importance in relation to the ageing process and prostatic disease in the elderly.

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Recovery of cutaneous immune responsiveness after PUVA therapy

S.I.WHITE, P.S.FRIEDMANN, C.MOSS AND J.M.SIMPSON*

Department of Dermatology, and *Department of Medical Statistics, University of Newcastle upon Tyne,
Newcastle upon Tyne NE1 4LP, U.K.

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SUMMARY

We studied sensitization to dinitrochlorobenzene (DNCB) in psoriatic patients before, during and 2,4, and 6 weeks after a course of photochemotherapy (PUVA). The inhibition of sensitivity observed during PUVA was completely reversed after 6 weeks. Thus the PUVA-mediated inhibition of cutaneous immune responsiveness is of short duration. However, whether there are long-term sequelae remains to be established.

Therapeutic photochemotherapy of psoriasis with 8-methoxypsoralen and ultraviolet A radiation (PUVA) causes a significant impairment of susceptibility to sensitization by 2,4-dinitrochlorobenzene (DNCB).[1,2] This inhibition is mainly a local cutaneous effect on the induction phase of the response, probably due to effects upon Langerhans cells,[3] but in addition, there is a local inhibition of the expression phase.[4] The significance of this immune impairment is not clear. In experimental animals, UVB irradiation produces an impairment of immune function[5,6] which has been clearly implicated in the mechanisms of subsequent tumour formation.[7,8] PUVA is also carcinogenic in animals[9] although whether immune mechanisms are important in this remains to be determined. It has been claimed that in man, PUVA therapy predisposes to the formation of skin cancers.[10] However, the biological importance of PUVA-induced immune non-responsiveness is likely to be related to its duration. We have, therefore, studied patients with psoriasis after completion of a course of PUVA therapy to determine whether and when their susceptibility to sensitization by DNCB returns to normal.

METHODS

Seventy three patients with psoriasis were divided randomly into five groups. The age range and sex ratio were similar in all groups. All patients gave informed consent. In order to follow the time course of changes in susceptibility, the groups of patients were sensitized at different times

Correspondence: Dr S.I. White, Department of Dermatology, University of Glasgow, Glasgow G12 8QQ, U.K.

TABLE 1. Proportions of patients sensitized to DNCB before, during and after PUVA

Group	Time of sensitization in relation to PUVA course	Number of patients tested	Number of patients sensitized (%)
1	pre-PUVA	23	22 (96%)
2	during PUVA	22	11 (50%)
3	2 weeks after PUVA	9	9 (100%)
4	4 weeks after PUVA	12	12 (100%)
5	6 weeks after PUVA	7	7 (100%)

in relation to the course of PUVA therapy (Table 1). All patients except Group 1 (pre-PUVA) had received thrice weekly PUVA according to a standard regimen.[11] Subjects in Group 2 received the initial dose of DNCB after 3–4 weeks (9–12 exposures). Treatment was continued until the psoriasis had cleared.

Induction of sensitivity with DNCB

All subjects were sensitized with 500 μg of DNCB in acetone, applied to a circle 3 cm in diameter on the volar aspect of the forearm and occluded for 48 h. We had found previously that this was the lowest dose that sensitized 100% of both healthy individuals and subjects with psoriasis.[12]

Challenge with DNCB

In patients in all groups except in Group 2 (sensitized during PUVA therapy), challenge doses were applied 4 weeks after sensitization. Patients in Group 2 were challenged 4 weeks after completion of PUVA to minimize residual local anti-inflammatory effects. Three or four challenge doses ranging from 25–3·216 μg were applied on standard foil-backed patches 10 mm in diameter (Al-test) held in place for 24 h on the volar surface of the contralateral forearm. The responses were determined 48 h after challenge as increase in skinfold thickness, measured with Harpenden skin calipers from which one spring had been removed to reduce compression.[13]

Statistical analysis

Mean responses were plotted against log challenge dose for each group, and the dose-response curves were compared using analysis of variance. As some patients received only three of the four challenge doses, the design is slightly unbalanced; therefore, the analysis was carried out using the statistical computing system GLIM. Straight lines were fitted to the dose-response curves, as there was no significant deviation from linearity in the effect of challenge dose. The slopes of the lines were compared and for those lines found to be parallel, the intercepts were then compared. When slopes differed significantly, responses were compared separately at each challenge dose by Student's *t*-test.

RESULTS

Proportions of patients sensitized

Of the patients sensitized before exposure to PUVA (Group 1), 22 out of 23 (96%) became sensitized; whereas of those sensitized during PUVA therapy (Group 2), only 11 out of 22 (50%) showed reactivity. In Group 2 there was no significant difference between the total UVA dosage

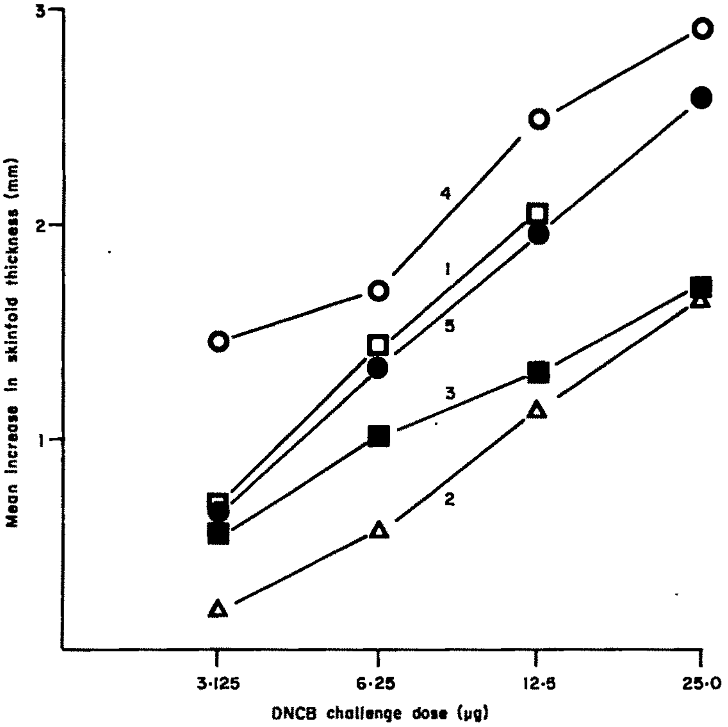

FIGURE 1. Challenge dose-response curves for the five groups of patients tested. Group 1 sensitized before PUVA (□——□), Group 2 during PUVA (△——△), Group 3 2 weeks after PUVA (■——■), Group 4 4 weeks after PUVA (○——○) and Group 5 6 weeks after PUVA (●——●).

received by subjects who became sensitized and those who did not; mean ± SD $31{\cdot}1 \pm 11{\cdot}2$ J/cm^2 and $31{\cdot}8 \pm 10{\cdot}7$ J/cm^2, respectively ($P > 0{\cdot}1$). All patients sensitized after cessation of PUVA therapy (Groups 3, 4 and 5) became sensitized. The proportion of patients sensitized was thus significantly lower during PUVA therapy than before or after PUVA ($P < 0{\cdot}0001$; χ^2 test).

Degree of sensitivity

PUVA therapy was found to have a significant effect on the degree of reactivity of those patients who became sensitized. The slopes of the dose-response curves for the five groups were significantly different ($P < 0{\cdot}005$) (Fig. 1). For Groups 2 and 3, sensitized during and 2 weeks after PUVA respectively, the slopes of the lines were significantly less than that for Group 1, sensitized before PUVA. The slope for Group 4, sensitized 4 weeks after PUVA was not significantly different from that for Group 1.

Group 2 gave significantly lower responses than Group 1 at challenge doses of 6·25 and 12·5 µg, ($P < 0{\cdot}01$), but not at the lowest dose. The position of the dose-response curve for Group 3 was not significantly different from that for Group 2. There were no significant differences in the slopes or positions of the curves for Groups 1, 4 and 5, pre-PUVA and 4 and 6 weeks post-PUVA, respectively. Although at 4 weeks after PUVA treatment sensitivity appeared greater than normal, this difference was not statistically significant. There was a large variability in response between subjects within each group, so larger numbers of subjects would be required for a difference of this magnitude (mean 0·46 mm) to be significant.

DISCUSSION

The main observation presented here is that PUVA-mediated impairment of delayed hypersensitivity is a rather short-term effect. Susceptibility to sensitization was recovering after only 2 weeks and was completely restored by 6 weeks after cessation of PUVA. This is shown by the return to normal of both the proportion of patients sensitized and their degree of reactivity. From our previous observations on normal responses to DNCB[12] and amplified responses in contact eczema[14], we concluded that the position of the challenge dose-response curve reflects the degree of sensitivity induced, while the slope of the curve reflects local factors acting in the expression phase of the response. On this basis, PUVA appears to inhibit both the induction and expression of contact hypersensitivity to DNCB, but both effects were of short duration and were no longer apparent 6 weeks after PUVA.

We have shown previously that the main effect of PUVA on cutaneous delayed hypersensitivity is a local inhibition of the induction phase.[4] This effect is probably a result of damage to epidermal Langerhans cells.[3] These cells, which are essential for the induction of contact hypersensitivity, lose their membrane markers of adenosine triphosphatase (ATPase)[3,15] and HLA-DR antigens (Parker and Friedmann, personal observation) within 1–2 weeks of commencement of PUVA therapy. After cessation of PUVA, numbers of Langerhans cells in the epidermis return to normal over a 3-week period.[3] This time course parallels the return of susceptibility to sensitization by DNCB. This could also explain why attempts to avert sensitization by agents such as topical nitrogen mustard by pre-treatment with PUVA have been unsuccessful.[16]

This, we have shown that the profound immune impairment produced by a single short course of PUVA is completely and rapidly reversible. Whether such an immunological 'window' has any long term effects will require further study.

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Topical 5-fluorouracil solution in the treatment of warts—clinical experience and percutaneous absorption

H.SENFF, D.REINEL, C.MATTHIES* AND D.WITTS†

Department of Dermatology, Federal Armed Forces Hospital, Hamburg, West Germany; *Department of Clinical Research, Hermal, Reinbek, West Germany and †Clinical Unit, Simbec Research Ltd., Merthyr Tydfil, Mid Glamorgan, Wales, U.K.

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SUMMARY

Disseminated warts in six patients were pre-treated with topical 5-fluorouracil solution three times daily for 5 consecutive days. Curettage was then carried out under local anaesthesia. During pre-treatment less than 0·1% of the dose applied was absorbed through the skin. The warts had not reappeared in 5 of the 6 patients at follow-up 1 year later.

Warts are the result of infection with papilloma viruses. The presence of the virus causes an increase in the rate of division of the epidermal cells and in the production of excess and abnormal keratohyalin granules. This is manifested histologically as acanthosis, papillomatosis and hyperkeratosis of the epidermis.

The anti-metabolite 5-fluorouracil (5-FU) is established in the treatment of such neoplasms. Through its ability to inhibit DNA and RNA synthesis, 5-FU inhibits cell proliferation and restricts spreading of the virus.[1]

Topically applied Verrumal® solution (containing 5 mg 5-FU, 100 mg salicylic acid and 80 mg dimethylsulfoxide per g, in a film forming base) has been used for the treatment of warts in Germany for many years.[2,3] The therapeutic effect has been acknowledged by some workers[4,5] but others have expressed concern about the risks of absorption of 5-FU and benefits of this mode of treatment.[6,7]

A single and multiple dose study of 5-FU in the treatment of warts in six patients was, therefore, carried out to assess the therapeutic effect, and measure the amount of 5-FU absorbed percutaneously during treatment of large areas of warts.

METHODS

The study was of an open design and consisted of a single application of Verrumal® solution (Hermal, Reinbeyk, West Germany) followed, after a period of at least 10 days without

Correspondence: Dr H.Senff, Bundeswehrkrankenhaus, Hautabteilung, Lesserstraße 180, 2000 Hamburg 70, West Germany.

application, by multiple applications in the same patients. Following application, urine was collected for 5-FU measurements during both phases of the study. Blood samples for 5-FU measurements were taken only during the multiple application phase. The study conformed to the Declaration of Helsinki and was approved by an independent ethics committee.

Six adult volunteers (mean age 21 years, range 19 years to 24 years) who had disseminated warts covering at least 25 cm^2 of the skin surface were included in the study. Three had simple plantar warts, two had warts on their hands and one patient had interdigital warts on his left foot as well as on his hands. The warts had been present for a mean of 14 months (range 10–22 months). Prior to inclusion in the study, four patients had previously been treated by various methods, without success. A full medical assessment, including a physical examination, clinical biochemistry, haematology and urinalysis, indicated that all the subjects were in good health before and after the study.

Single application phase

Approximately 0·6–1 g of the Verrumal® solution (containing 0·5% 5-FU and 10% salicyclic acid) was applied to the warts. Total urine collections were made for 24 h before application, and for 0–8 h, 8–24 h, 24–48 h and 48–72 h after application.

Multiple application phase

Two applications of Verrumal® were made with an interval of 8 h. Then applications were made at 8 h intervals, three times daily for 4 days. On the final study day a single application was made. All applications were carried out by the same physician.

Blood samples were collected immediately before the first application, and before the 4th (24 h), and 15th (112 h) applications, and 24, 48 and 72 h after the last (15th) application. Total urine collections were made during the following periods after the first application: 0–16, 16–40, 40–64, 64–88, 88–112 h, and 24, 48 and 72 h after the last application.

During each application the area immediately surrounding the warts was covered, however, a band of clinically unaffected skin around the warts was always treated.[8] To enhance the penetration of 5-FU into the warts, the horny material was removed once-daily with a nail-file.

Curettage of the warts under local anaesthetic was performed immediately after the 15th application of the Verrumal® solution.

The Verrumal® bottles were weighed before and after each application and the amount of Verrumal® applied was calculated. The concentrations of 5-FU in urine and plasma were estimated by a modification of the method of Kok *et al.*[9] The total amount of 5-FU excreted was calculated as the sum of the concentrations of 5-FU multiplied by the total volume of urine collected over each time period. An estimate of the amount of 5-FU absorbed through the skin was made by assuming that urinary 5-FU represented 11% of the available dose.[10,11]

RESULTS

Plasma 5-FU measurements

Plasma concentrations of 5-FU varied markedly between individuals and did not appear to change in a consistent pattern with time (Table 1). The variation was such that it was not possible to define the kinetics of elimination of 5-FU from the plasma. Comparison with the amounts of 5-FU excreted in the urine over the same time period (Table 2) demonstrates that the plasma concentrations of 5-FU were a poor guide to the extent of absorption in our patients. These findings are similar to those of Sadee and Wong.[12]

TABLE 1. Plasma concentration of 5-FU after application of Verrumal® solution to warts

Time after first application (h)	Plasma 5-FU ng/ml in subject no.					
	1	2	3	4	5	6
Before application	ND	ND	ND	ND	ND	ND
24	ND	1·892	1·939	3·456	0·550	4·246
112	ND	1·455	ND	ND	ND	1·058
136	ND	2·461	ND	ND	0·850	2·450
160	ND	0·809	0·752	ND	1·539	3·233
184	ND	0·701	1·295	ND	ND	2·036

ND = not detected

Urine 5-FU measurements

Urine 5-FU levels are listed in Table 2.

The calculated total median percent absorption of 5-FU was 0·085% after a single application and 0·035% after multiple applications.

The lower limit of accurate detection of the assay for 5-FU in urine was approximately 0·5 ng/ml. All urine samples containing less than 0·5 ng/ml have therefore been reported 'as not detected'. In order to test the 'worst case' hypothesis these samples were reworked as if each contained 0·499 ng/ml. Theoretically, under these circumstances, a median of 0·41% and 0·12% could have been absorbed after single and multiple applications, respectively.

TABLE 2. Urine concentrations of 5-FU after application of Verrumal® solution to warts

Collection period; h after application	Urine 5-FU ng/ml in subject no.					
	1	2	3	4	5	6
Single application						
Before application	ND	ND	ND	ND	ND	ND
0–8	ND	0·571	ND	ND	ND	ND
8–24	ND	ND	0·669	ND	ND	ND
24–48	ND	0·754	ND	ND	0·661	ND
48–72	ND	ND	ND	ND	ND	ND
Multiple application						
Before application	ND	ND	ND	ND	ND	ND
0–16	ND	ND	0·924	ND	ND	ND
16–40	ND	ND	0·658	ND	ND	ND
40–64	ND	ND	ND	ND	ND	ND
64–88	0·509	ND	0·652	ND	0·705	ND
88–112	1·037	4·754	ND	0·634	0·858	ND
112–120	ND	6·225	ND	ND	0·643	ND
120–136	0·610	0·638	1·339	0·551	ND	ND
136–160	ND	ND	1·280	ND	ND	ND
160–184	ND	ND	ND	ND	ND	ND

TABLE 3. Summary of 5-FU absorption data after application of Verrumal® to warts

	Subject no.						
	1	2	3	4	5	6	Median
Single application							
Weight of 5-FU applied (mg)	5·60	2·90	3·00	4·70	4·10	4·85	4·40
Total amount of 5-FU excreted (μg)	0	1·42	0·64	0	0·77	0	0·32
Calculated amount of 5-FU absorbed (μg)	0	12·88	5·78	0	7·03	0	2·89
Percentage absorption	0	0·44	0·19	0	0·17	0	0·085
Multiple application							
Weight of 5-FU applied (mg)	72·95	54·40	55·15	55·15	53·10	67·05	55·15
Total amount of 5-FU in plasma (ng)	0	7·31	3·98	3·46	2·94	13·01	3·77
Total amount of 5-FU excreted (μg)	2·42	11·85	8·35	0·68	2·31	0	2·37
Calculated amount of 5-FU absorbed (μg)	22·02	107·72	75·92	6·17	20·95	0	21·49
Percentage absorption	0·03	0·20	0·14	0·01	0·04	0	0·035

There was no apparent correlation between the total dose applied and the amount of 5-FU which subsequently appeared in the urine following either single or multiple application. No accumulation appeared to occur.

The main parameters are listed in Table 3.

Clinical results

All patients showed a whitening of the skin (induced by the salicylic acid) and a softening of the horny material, from the second day of treatment.

After the 15th application the warts were well prepared for curettage. The infected skin could easily be removed down to the corium leaving most of the surrounding skin undamaged. Figure 1 shows the infected area in a patient with interdigital warts before treatment. Figure 2 shows the effect of Verrumal® solution after the 15th application. The treated area 14 days after curettage is shown in Figure 3. Similar therapeutic results were obtained in the other five patients.

All patients were re-examined 3 months and 1 year after treatment. One patient had developed new warts, but at different locations to those previously treated; another patient had a recurrence of solitary warts at the original site. All of these were successfully treated as before.

There were no drug related adverse effects during treatment.

DISCUSSION

In this study all six patients were treated as in-patients due to the disabling effect of the warts on their hands and feet. In our experience the solution must be applied at least three times daily for 5 consecutive days if there is an extended disseminated area of warts. Curettage should be

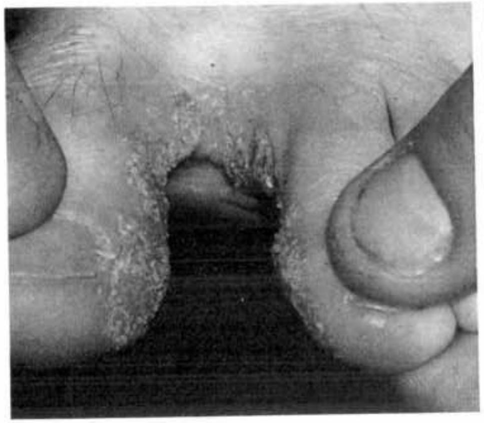

FIGURE 1. Interdigital warts before treatment.

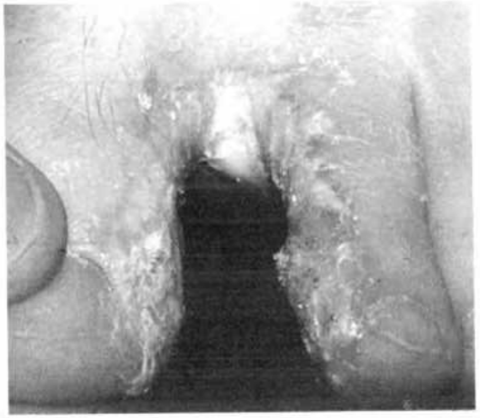

FIGURE 2. Interdigital warts after 15th application of Verrumal® solution.

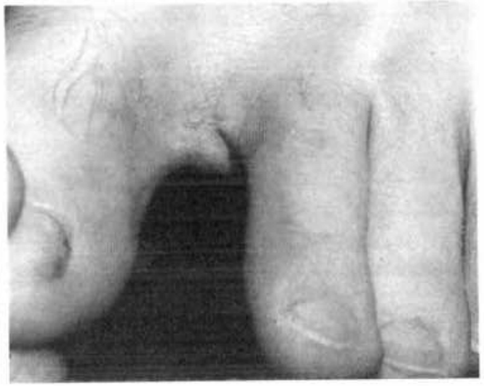

FIGURE 3. Clinical features of the treated area 14 days after curettage.

performed on the fifth day. There should be no interval between pre-treatment with 5-FU solution and the excochleation of large areas of warts, because of the short duration of the softening effect of the treatment. Following pre-treatment, the warts could easily be removed with a curette. In contrast to the findings of Kaufmann and Landes[7] who reported that 5-FU treatment after wart-curettage did not minimize relapse, our patients benefited from treatment with 5-FU solution before curettage. This combination of treatments may prove to be a useful supplement to the available treatments for large infected areas, supporting the experience of Jablonska (personal communication), who suggested that a combination of two or more treatments might be the most effective.

The main aim of the present study was to measure the absorption of 5-FU applied to warts. Therefore, patients with large areas of warts for whom curettage was intended were studied. The benefits of pre-treatment with Verrumal are worthy of note. The infected skin was softened to the same extent as after occlusive application of salicylic acid plasters over 10 days, which facilitated curettage. No scars were observed after treatment. For out-patients the advantages of Verrumal are painless application and also the possibility of monitoring the patient's compliance. In minor infections operative intervention can be avoided in many cases.[13]

We conclude that 5-FU solution is useful for the treatment of warts in out-patients and also is a valuable pre-operative treatment for extensive infections. The solution can be applied safely as the extent of systemic absorption appears to be very small, especially as the total dose in out-patients is normally less than 1 g (equivalent to 5 mg 5-FU).

ACKNOWLEDGMENTS

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Recalcitrant acne vulgaris. Clinical, biochemical and microbiological investigation of patients not responding to antibiotic treatment

E.ANNE EADY, J.H.COVE, J.BLAKE *, K.T.HOLLAND AND W.J. CUNLIFFE *

Department of Microbiology, University of Leeds and * Department of Dermatology, Leeds General Infirmary, Leeds, U.K.

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SUMMARY

A small, but clinically significant proportion of acne patients fail to respond adequately to antibiotic therapy. All non-responding acne patients attending the Leeds General Infirmary between September 1985 and April 1986 (49 out of a total of 610 patients; 8%) were investigated with respect to changes in their acne grade, microbial flora and sebum excretion rate. They were compared with 22 age and sex matched untreated control subjects. It was found that in 65% of non-responding patients there was no microbiological abnormality, in 16% there was evidence of Gram-negative folliculitis and 20% carried predominantly antibiotic resistant propionibacteria compared with only 5% of untreated controls. There was a significant association between erythromycin therapy and the isolation of erythromycin resistant propionibacteria ($P < 0{\cdot}001$). A causal link, however, has yet to be established between carriage of antibiotic resistant propionibacteria and failure to respond to antibiotic therapy. Our results show that for most patients with recalcitrant acne a non-microbiological explanation must be sought for the lack of therapeutic success. The mean sebum excretion rate (SER) of the non-responding patients was significantly higher than that of matched untreated acne patients ($P < 0{\cdot}001$). A majority of non-responders (69%) had an SER above the upper 95% confidence limit of the control mean. The SER may affect treatment efficacy by influencing the antibiotic concentration within the pilosebaceous ducts.

Cutaneous propionibacteria are strongly implicated in the pathogenesis of acne vulgaris, and agents which reduce the numbers of *Propionibacterium acnes* are therapeutic. Oral antibiotics are the treatment of choice for the majority of patients with clinical acne ('acne major') and the

Correspondence: Dr E. Anne Eady, Department of Microbiology, University of Leeds, Leeds LS2 9JT, U.K.

degree of improvement is usually good. However, a number of patients fail to respond or do not respond adequately to antibiotic therapy[1–3]. Various reasons have been put forward to explain this therapeutic failure including antibiotic resistant propionibacteria, abnormal pharmacokinetics or poor patient compliance.

Early attempts to relate therapeutic failure to antibiotic resistance in propionibacteria were unsuccessful[2] and it seemed that *P.acnes* was not capable of developing resistance despite more than 20 years extensive use of oral antibiotics in acne therapy. First reports of antibiotic resistant propionibacteria isolated from acne patients began to appear in the late nineteen seventies[4–6] although occasional strains resistant to tetracycline or erythromycin or both had been reported earlier than this amongst clinical isolates from a variety of non-cutaneous sources.[7–10]

In 1983 Leyden *et al.*[11] reported for the first time in the U.S.A. a correlation between lack of therapeutic response and antibiotic resistant propionibacteria in acne patients. A similar study carried out in the U.K.[12] also detected the presence of resistant propionibacteria in non-responding acne patients, although the incidence was much lower than in the American study.

The objectives of the present study were firstly to re-examine the extent of antibiotic resistance in propionibacteria in the U.K., in both treated and untreated subjects, and secondly, to determine whether such resistance could be responsible for the lack of improvement of some antibiotic treated patients. Additional factors including sebum excretion rate and incidence of Gram-negative folliculitis were also investigated in order to uncover other potential explanations for the lack of therapeutic response in these patients.

METHODS

Subjects

Forty-nine non-responding acne patients and 22 age and sex matched untreated controls were included in the study (Table 1). The patients had been on antibiotic therapy for a minimum of 4 months and the majority were using benzoyl peroxide topically. Subjects were defined as non-responding if their acne grades on three consecutive clinic visits were unchanged. The usual interval between visits was 12 weeks. The 49 non-responding acne patients represented 8% of the total number of similarly treated patients (610) attending the acne clinic between September 1985 and April 1986. The control subjects, recruited from the general population, had received no antibiotic therapy in the 12 months prior to sampling and had never been treated with antibiotics for acne.

TABLE 1. Details of patients and controls included in the study.

Treatment group	Number of subjects	Age (years) mean (range)	Male: female ratio	Sample site ratio (face:back)*
Erythromycin	15			
Tetracycline	7			
Minocycline	27			
Total	49	19·6 (13–28)	3·5:1	0·8:1
Untreated controls	22	20·0 (14–28)	3·4:1	0·7:1

* Patients were sampled from the worst affected site (the face or back). The same proportions of subjects were sampled from each site in the treated and untreated groups.

For measurements of sebum excretion, 48 untreated acne patients, matched for age, sex and acne grade were used as controls.

Microbiology

Sample collection. Bacteria and yeasts were obtained from the skin surface by a detergent scrub technique.[13] Viable counts were obtained by plating out serial 10-fold dilutions of the wash fluid onto Brain Heart Infusion agar (incubated anaerobically at 37°C for 6 days) for isolation of propionibacteria, onto Heated Blood agar incubated aerobically at 37°C for 48 h for isolation of staphylococci, and a novel milk-based medium[14] (incubated at 37°C for 10 days in a moist chamber) for detection of *Pityrosporum spp* . All patients were sampled from the worst affected site (the face or back).

Detection of antibiotic resistance. Antibiotic resistant propionibacteria were enumerated directly from skin by plating out undiluted wash fluid and a 10^{-2} dilution onto Isosensitest agar (Oxoid Ltd, Basingstoke, Hants, U.K.), containing, erythromycin, 0·5 μg/ml, clindamycin, 0·5 μg/ml, tetracycline, 5·0 μg/ml or minocycline 2·0 μg/ml. The antibiotics were obtained from Sigma Chemical Co (Poole, Dorset, U.K.) with the exception of clindamycin which was a gift from Upjohn Ltd (Crawley, West Sussex, U.K.). The concentrations of antibiotics used represent five times the minimum inhibitory concentration for 90% of isolates. The medium was supplemented with tryptophan, valine, leucine and cysteine (300 mg/l of each) to enhance the growth of propionibacteria and 2·0 μg/ml furazolidone (Sigma) to inhibit the growth of staphylococci.[15]

Sebum excretion rate

Sebum excretion rate was determined on the foreheads of the patients and controls using the method of Lookingbill and Cunliffe.[16]

Statistical analyses

The results were analysed using the χ^2 test with Yates' correction, or Student's *t*-test.

RESULTS

Microbial numbers

No significant difference was found between the numbers of staphylococci, propionibacteria or *Pityrosporum* species in patients and control subjects (Table 2), although there was a tendency for staphylococcal numbers to be raised and propionibacterial numbers to be depressed in the patients. Gram-negative rods were isolated from eight patients, five on minocycline, two on erythromycin and one on tetracycline, but not from the control subjects.

Antibiotic resistant propionibacteria

A majority of the non-responding acne patients (59%) and of the controls (95%) had no detectable antibiotic resistant propionibacteria. Erythromycin resistance (E^R) was the most frequently encountered; 39% of patients carried E^R or erythromycin-clindamycin resistant (E^RCD^R) propionibacteria compared with 5% of the controls ($P < 0{\cdot}01$) (Table 3). There was a strong association between erythromycin therapy and carriage of E^R strains; 67% of erythromycin treated patients harboured resistant strains, a significantly greater proportion than in the controls ($P < 0{\cdot}001$). A substantial number of the tetracycline and minocycline treated patients (27%) also harboured E^R or E^RCD^R propionibacteria, possibly acquired during

TABLE 2. Comparison of microbial densities in patients and control subjects

	Bacterial counts $\log_{10}$ colony forming units/cm^2 skin (mean ± SD)		
	Controls ($n=22$)	Patients ($n=49$)	P*
Staphylococci	3·21 ± 1·62	3·95 ± 1·35	NS
Propionibacteria	4·56 ± 2·14	3·74 ± 2·02	NS
Pityrosporum spp.***	2·72 ± 1·28	2·60 ± 1·35	NS
Gram-negative rods	0	2·89 ± 0·73**	

* Student's *t*-test; NS = Not significant; ** Mean ± SD for the eight patients in whom these organisms were detected. *** Data for 34 patients and 12 control subjects only.

a previous course of treatment with erythromycin. Case histories confirmed that seven of these nine patients had received a course of erythromycin for their acne in the past; the time since the last exposure varied from 1 month to over 3 years (mean 21 months).

In the erythromycin treated group, E^R propionibacteria were usually, but not always, a major component of the anaerobic flora (Fig. 1). In the tetracycline and minocycline treated patients E^R strains usually represented a small percentage of the total propionibacterial flora.

Tetracycline (Tet^R) and minocycline (Min^R) resistant propionibacteria were rarely encountered; 14% of patients and 0% of controls carried Tet^R strains and 3 patients carried Min^R strains. In only 3 patients was the Tet^R strain predominant.

Amongst the non-responding patients, the incidence of resistant strains was higher on the face (55%) than on the back (30%) and higher in females (64%) than males (34%) although the differences were not statistically significant. All the erythromycin treated females carried E^R strains and E^R strains were always predominant on the face, but not on the back in erythromycin treated patients.

Therapeutic failure was associated with carriage of predominantly resistant propionibacteria in only 20% of patients (Table 4). In a further 16%, lack of response was associated with the presence of Gram-negative rods. However, most patients (65%) had no microbiological abnormality which might have accounted for their poor response to treatment.

TABLE 3. Carriage of antibiotic resistant propionibacteria by patients and controls

Treatment group	Percentage of subjects carrying strains resistant to:			
	Tetracycline	Minocycline	Erythromycin	Clindamycin
Tetracycline	14	0	29	29
Minocycline	15	11	25	15
Erythromycin	14	0	67**	40*
Untreated controls	0	0	5	5

Significantly different from untreated controls (χ^2 test). * $P < 0·05$; ** $P < 0·001$.

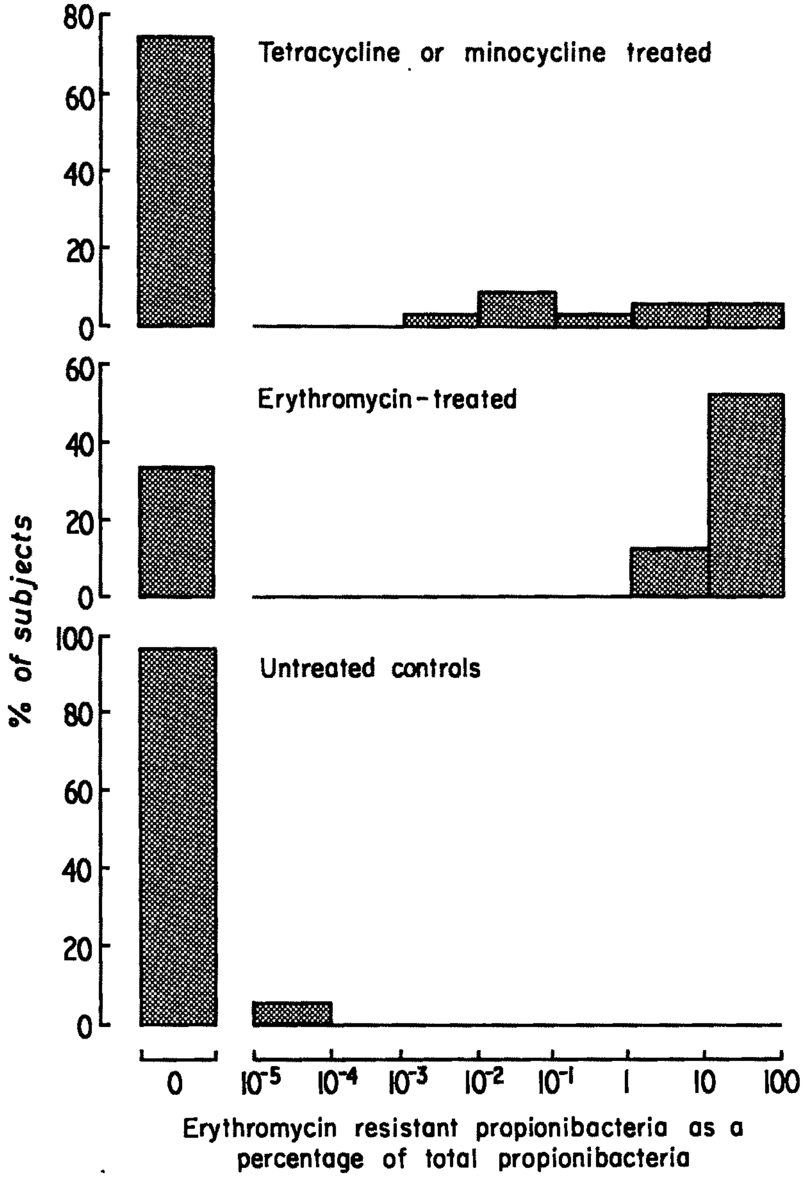

FIGURE 1. Frequency distributions of erythromycin resistant propionibacteria in patients and controls. The distributions in the tetracycline or minocycline treated patients and the erythromycin treated patients were significantly different from that in the control group ($P<0{\cdot}01$ and $P<0{\cdot}001$, respectively; χ^2 test).

Sebum excretion rate

The sebum excretion rate (SER) was significantly higher in the non-responding acne patients than in the untreated control acne patients ($P<0{\cdot}001$, Student's t-test). The mean SER of the patients (± SD) was $1{\cdot}68 \pm 0{\cdot}51 \mu g/cm^2/min$ (range 0·25–2·68 $\mu g/cm^2/min$) and that of the controls was $1{\cdot}25 \pm 0{\cdot}37$ $\mu g/cm^2/min$ (range 0·33–2·09 $\mu g/cm^2/min$). It was also found that 33

TABLE 4. Incidence of abnormal microbiological findings in non-responding patients

		Abnormal microbiology	
Treatment group	Normal microbiology	Gram-negative folliculitis	Predominantly resistant propionibacteria
Erythromycin ($n = 15$)	6	2*	8*
Minocycline ($n = 27$)	21	5	1
Tetracycline ($n = 7$)	5	1	1
Total ($n = 49$)	32 (65%)	8 (16%)	10 (20%)

* One patient had both resistant propionibacteria and evidence of Gram-negative folliculitis.

(69%) of the non-responding patients had an SER greater than the upper 95% confidence limit of the control mean.

Patient follow-up

When the results of the microbiological analyses became known to the clinician in charge of the patients (W.J.C.), the therapeutic regimens were modified accordingly. Thirty of the patients (61%) had by this time been treated with several different antibiotics without significant improvement. Of these, 12 (24%) were changed to 13-cis retinoic acid and all had a satisfactory outcome (Table 5). Three other patients who were also transferred to 13-cis retinoic acid therapy responded well. Patients with clinical and bacteriological evidence of a Gram-negative folliculitis were changed to trimethoprim or co-trimoxazole, with variable results. The remaining patients were changed either to trimethoprim or to another antibiotic, mainly because their acne was not sufficiently severe to merit 13-cis retinoic acid therapy. Again, the response was variable. Overall, 26% of patients failed to improve with the alternative therapy. Ten patients (26%) did not attend follow-up visits.

TABLE 5. Outcome of alternative therapies

	Alternative therapy				
	13-Cis retinoic acid	Trimethoprim	Other antibiotic	Miscellaneous	Total
Number of patients transferred	15	15	12	4	46*
Responders	15	7	4	3**	29
Non-responders	0	5	5	0	10
Lost to follow-up	0	3	3	1	7

* Three patients failed to attend follow-up microbiological tests, therefore no change of therapy could be initiated.

** One patient changed to Diane plus cyproterone acetate, one patient continued on same treatment and one patient was taken off all oral therapy.

DISCUSSION

A small, but clinically significant proportion (8%) of acne patients attending the dermatology department at Leeds fail to respond adequately to antibiotic therapy. The results of the present study suggest that there may be several different reasons for this lack of therapeutic response.

Our results are consistent with previous reports which have shown that in untreated subjects the incidence of antibiotic resistant propionibacteria is low, both in the U.K. and U.S.A.[5,12,17] The results of the present study and of others suggest that in the majority of cases there is a clear association between the emergence of erythromycin resistant propionibacteria and therapeutic use of the drug.[5,11] A similar situation appears to exist for clindamycin use and clindamycin resistance.[5,12,18]

The incorporation of furazolidone into isolation media inhibited the growth of coagulase-negative staphylococci.[15] This facilitated the detection without subculture of antibiotic resistant propionibacteria even when they constituted only a minor component of the skin flora. The method can be used routinely to screen large numbers of acne patients and to monitor changes in numbers of resistant organisms.

The present study is the first, to our knowledge, in which resistant organisms have been quantified as a proportion of the total propionibacterial flora. Individuals in whom resistant organisms comprised only a small fraction of the total flora would have been reported as possessing a sensitive flora had only predominant strains been tested. Our results clearly show that erythromycin resistance persists in a small proportion of the microbial flora for a long period after exposure to the drug has ceased (mean 21 months). This finding contrasts with studies in which only predominant strains were tested and which suggested that resistance is rapidly lost once therapy is discontinued.[5,11] Indeed, the long-term persistence of resistant strains might account for the reported isolation of such strains from 'untreated' control subjects[6] who may have been exposed to antibiotics many months prior to sampling.

Therapeutic failure was associated with carriage of predominantly antibiotic resistant propionibacteria in only 20% of the patients, most of whom had been treated with erythromycin. In the majority of patients the reason for their lack of response must be sought elsewhere. Eight patients (16%) had both clinical and microbiological evidence of Gram-negative folliculitis, a recognised complication of antibiotic therapy for acne.[19] Overgrowth of yeasts of the genus *Pityrosporum* was not found.

The non-responding patients did not show significantly lower numbers of propionibacteria compared with the control subjects. There is, however, considerable disagreement as to whether numbers of propionibacteria are reduced by antibiotic therapy,[20] so that this finding does not itself explain therapeutic failure. Serum antibiotic levels were only available for 10 of the erythromycin treated patients (0·007–1·0 μg/ml). The serum level in five patients was below the minimum inhibitory concentration for sensitive strains of *P.acnes* ($\leqslant$0·1 μg/ml). More important than the serum level is the skin level of antibiotic, which few workers have attempted to measure due to the technical difficulties involved. It is probable that unless skin levels exceed serum concentrations, lack of therapeutic response could be due in some cases to insufficient antibiotic reaching the target site. Low serum levels could be due to poor antibiotic absorption, however, deliberate non-compliance by patients may also occur. This may be particularly prevalent among antibiotic treated patients who perceive 13-cis retinoic acid as an alternative and potentially more powerful treatment. The sebum excretion rate is known to be correlated with the severity of acne[21] and it has been shown that patients with a high SER are more difficult to treat.[22] The SER of the non-responding patients was significantly higher than that of the control group. It is difficult to establish a causal link between a high SER and failure to respond to antibiotic therapy, particularly since many acne patients with elevated SER levels respond satisfactorily to antibiotic therapy. It is, however, possible that if sebum is the main route of delivery of antibiotic to the skin, SER could regulate the concentration of the drug *in vivo*. Perhaps other as yet uninvestigated physiological factors can affect antibiotic activity in the skin. For example, the effective concentration of erythromycin would be diminished *in vivo* due to its instability at the acidic pH found normally on human skin. The extent of this reduction would vary from subject to subject depending on the pH of the skin.

Antibiotic resistant propionibacteria seem to have emerged at about the same time in both the U.K. and the U.S.A. Differences in the incidence of particular resistance determinants probably reflect different antibiotic usage policies. Until more is known about the stability and mode of spread of resistance and the relationship, if any, between resistance and therapeutic failure, it is impossible to say whether the position of antibiotics as the mainstays of acne therapy is under threat. The carriage of resistant propionibacteria by patients responding well to antibiotic therapy requires investigation. The majority of non-responding patients in the present study had normal microbiology and the reason for their lack of response must lie elsewhere. Since acne is predominantly an inflammatory disease, the possibility of an abnormal immune response should be considered.

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Comparison of a hydrocolloid dressing and paraffin gauze in the treatment of venous ulcers

S.E.HANDFIELD-JONES, C.E.H.GRATTAN, ROSEMARY A.SIMPSON* AND C.T.C.KENNEDY

Departments of Dermatology and * Microbiology, Bristol Royal Infirmary, Bristol BS2 8HW, U.K.

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SUMMARY

In-patients with venous leg ulcers showed increased healing rates with a hydrocolloid dressing (Granuflex®), as compared with a traditional paraffin gauze dressing (Jelonet®), in a randomized sequential crossover trial, although the difference was not statistically significant.

Bacteriological studies showed that the hydrocolloid dressing favoured growth of both aerobes and anaerobes in significantly greater numbers compared with paraffin gauze. Heavy bacterial colonization had no effect on the healing rates of the ulcers.

Granuflex is said to produce an anaerobic environment—survival of *Pseudomonas aeruginosa*, a strict aerobe, under Granuflex dressings, makes this seem unlikely.

Various advantages have been claimed for the new generation of hydrocolloid dressings in the management of wounds and leg ulcers. These include the production of a moist environment at the wound surface, prevention of external bacterial colonization, better healing and improved patient acceptability and comfort. *In vitro* studies have shown that relatively low oxygen tension stimulates angiogenesis and fibroblast and epidermal cell turnover[1] and would, therefore, be expected to provide good conditions for wound healing. We, therefore, compared an oxygen impermeable hydrocolloid dressing (Granuflex,® Squibb Surgicare) with a conventional paraffin gauze dressing (Jelonet,® Smith & Nephew) with regard to healing rate, bacterial counts and patient acceptability to determine whether the new hydrocolloid dressing offers an advantage in the management of venous leg ulcers and how the anaerobic environment affects the bacterial flora.

METHODS

Ten venous ulcers in eight female patients (mean age 77 years, range 65–90 years) were studied. Following an initial preparatory period of 1 week for cleaning and debridement, the ulcers were randomized to receive either Granuflex® or Jelonet® for 3 weeks, followed by cross-over to the alternative dressing for a further 3 weeks. All patients were in hospital throughout the study and received the same nursing care, leg elevation and compression bandaging. The ulcers were cleaned with sterile normal saline at dressing changes, which were performed daily during treatment with Jelonet,® and at intervals dictated by the leakage of wound exudate with Granuflex®. Topical antimicrobial preparations were not used at any stage in the study.

Correspondence: Dr C.T.C.Kennedy.

Ulcer outlines were traced at the beginning of the trial and at weekly intervals until the end, and the surface areas were calculated using computer assisted planimetry.

Ulcers were swabbed at weekly intervals by a standard technique using a saline moistened alginate swab. Micro-organisms were eluted into dilute Ringer's solution, and inoculated onto culture media, using a spiral plater (Don Whitley Ltd., Toddington, Beds, U.K.). Counts were made after 48 h incubation on 5% horse blood agar and MacConkey bile-salt lactose agar (cultured anaerobically at 37°C), 5% horse blood agar containing neomycin 70 μg/ml (cultured aerobically at 37°C), and Sabouraud agar (incubated at 30°C, for enumeration of yeasts).

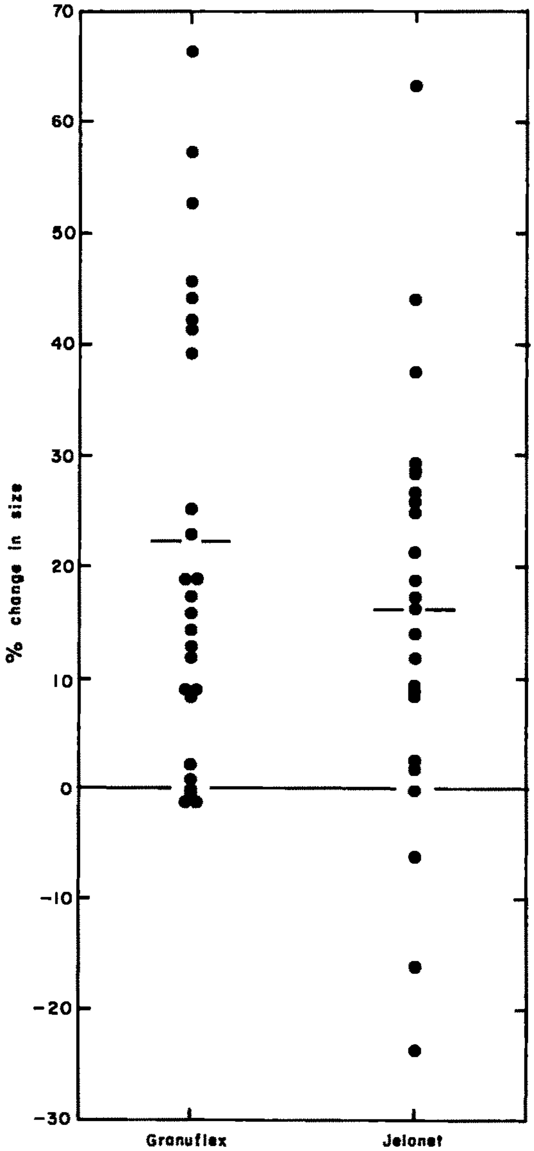

FIGURE 1. Percentage changes in ulcer size with Granuflex® and Jelonet®. Each point represents the change in one ulcer in 1 week. Bars are means. Negative values indicate enlargement of the ulcer.

RESULTS

Healing rate

All ulcers had decreased in surface area by the end of each 3-week dressing period. To allow for initial variation in ulcer size, the results are expressed as percentage change in ulcer size (Fig. 1). The healing rate of the ulcers was slightly faster when treated with Granuflex® (mean 22% reduction in size per week) than with Jelonet® (mean 17% reduction per week), but this difference was not statistically significant ($P > 0{\cdot}05$; paired *t*-test). Four ulcers showed a temporary increase in size, but this was never sustained over 2 consecutive weeks. Deterioration was more marked with paraffin gauze than with the hydrocolloid dressing.

Most patients thought that Granuflex® was more comfortable than Jelonet®, and dressing changes were less frequent with Granuflex®. One patient was withdrawn because of eczema below the Granuflex® dressing, but subsequent patch tests were negative.

Bacteriology

Two patients (3 ulcers) were excluded from the microbiological analysis because they were receiving concurrent systemic antibiotic therapy for respiratory tract infections.

In five out of the seven ulcers studied, counts of aerobic organisms were significantly higher with Granuflex than with Jelonet with up to a 100-fold difference within the same ulcer ($P < 0{\cdot}02$, paired *t*-test). Anaerobes were present in higher numbers beneath Granuflex in six out of the seven ulcers ($P < 0{\cdot}01$, paired *t*-test). No particular organism was favoured. In three patients, ulcers grew *Pseudomonas aeruginosa* beneath Granuflex dressings; in one patient *Ps. aeruginosa* counts increased throughout the 3 weeks Granuflex was used. Yeasts were not seen in any significant numbers.

Despite heavy bacterial growth there was never any clinical evidence of infection, and ulcers showing high bacterial counts improved as rapidly as those with lower counts.

DISCUSSION

Our results show that healing occurred with both dressings and although the healing rate was greater with Granuflex® the difference was not statistically significant.

The high bacterial counts in ulcers dressed with Granuflex® had no adverse effects, supporting the clinical impression that bacterial colonization, however heavy, is not detrimental. The persistence of *Pseudomonas aeruginosa,* a strict aerobe, suggests that the environment under Granuflex is not truly anaerobic.

Hydrocolloid dressings offer a satisfactory treatment for venous ulcers, but it should not be forgotten that conventional dressings also promote healing when used with adequate elevation and support. Patients should be assessed individually to decide the most suitable and cost-effective dressing, taking into consideration frequency of dressing changes, healing rates and patient comfort.

ACKNOWLEDGMENT

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The importance of the coal tar bath in the Ingram treatment of psoriasis. Evaluation by evaporimetry and laser Doppler flowmetry

T.FRÖDIN, M.SKOGH AND L.MOLIN

Department of Dermatology, University Hospital, Linköping, Sweden

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SUMMARY

The contribution of the tar component to the efficacy of the Ingram regimen in the treatment of psoriasis was assessed in 11 patients with symmetrical lesions on the upper extremities. One arm was immersed in an oil emulsion bath and the other in a coal tar bath. Whole body ultraviolet irradiation followed, and dithranol paste was applied to all lesions. Healing was assessed clinically at weekly intervals, and was found to parallel the normalization of transepidermal water loss (TEWL) as determined by evaporimetry, and the dermal blood flow as evaluated by laser Doppler flowmetry. Results obtained with coal tar baths were not significantly different from those obtained with oil emulsion. We conclude that coal tar bath additive has no advantage over oil emulsion in the Ingram regimen.

Dithranol has stood the test of time in the treatment of psoriasis, and it is used extensively in Europe as an ingredient in various topical regimens. Ingram's combination of coal tar bath, ultraviolet radiation (UVB), and dithranol in a stiff paste is highly efficient in clearing psoriasis.[1] The three components of this regimen were not evaluated separately until 1966, when Bowers[2] cast doubt on the value of coal tar. Nevertheless, the Ingram regimen gives such satisfactory results that it has usually been strictly and unquestioningly adhered to. However, there are good reasons for simplifying methods used in treating psoriasis, not least with regard to time and cost.

The aim of the present study was to evaluate the importance of the tar component in the Ingram regimen, following the healing process by clinical assessment, evaporimetry and laser Doppler flowmetry. Evaporimetry can be used to study epidermal barrier function,[3] which is disturbed in inflammatory skin conditions such as psoriasis,[4] and has been used in monitoring the healing process of this disease.[5] The microcirculatory component of inflammation can be assessed conveniently and non-invasively by laser Doppler flowmetry, and this method has been used for studying cutaneous blood flow in psoriasis lesions and the influence of treatment upon this.[6,7]

Correspondence: Thomas Frödin, Department of Dermatology, University Hospital, S-581 85 Linköping, Sweden.

METHODS

Patients

Eleven in-patients (two women and nine men, aged 15–72 years) with stable plaque psoriasis took part in the study. All were to be treated by the Ingram method, and had given their informed consent. The criterion for inclusion in the study was the presence of comparable and symmetrical plaques on both arms. No other treatment apart from topical emollients was allowed during the week preceding the start of the study.

Treatment

Most patients received treatment for 5 days, followed by 2 days without treatment, over a 4-week period. Each morning started with a 20 min hot bath (300 l) to which had been added 300 ml of spiritus carbonis detergens (Apoteksbolaget, Sweden; roughly corresponding to liquor picis carbonis, BP) (six patients), or 20 ml of oil emulsion (4% Hostaphat in liquid paraffin, ACO, Sweden) (five patients).

During the bath the right arm was immersed in a separate container with the alternative emulsion or mixture. After towel drying the patient immediately received UVB irradiation (Waldmann UV 1000 cabin equipped with Sylvania UV21 tubes), the exposure being empirically judged to give a minimum erythema dose (MED). Dithranol dispersed in standard Lassar's paste, initially at a concentration of 0·1%, and later if possible 0·4%, was then applied to each plaque. In the late afternoon white soft paraffin with 2% salicylic acid was applied to each lesion.

Measurements

Two psoriasis lesions, one on each arm and comparable in size, site, and clinical state were chosen. These were examined before starting treatment and then regularly once weekly for 4 weeks. On each occasion the two lesions were classified according to clinical criteria and measured for transepidermal water loss (TEWL) and dermal blood flow. One patient left the study after the first week, and two others were not examined at week 4 because they had already been discharged healed. An adjacent area of unaffected skin was used as a control. All measurements were made in the morning before bathing, with the patient lying down comfortably in a quiet room. Thus, in each patient, two lesions were studied during the course of a modified Ingram treatment regimen, all details being identical except that one lesion was treated in the traditional way whereas in the other the tar component was replaced by oil emulsion.

Clinically the lesions were assessed with regard to erythema, palpable infiltration, and desquamation, on a 3-point scale (0 = normal, 1 = slightly abnormal, 2 = highly abnormal). The scores for each feature were added up, giving a maximum total score of 6. Photographs were taken each week. At the end of the study the paired slides were arranged at random and shown to members of the medical staff of the department, who were asked to judge which side was best.

The transepidermal water loss (TEWL) was measured using a prototype of the EPl Evaporimeter (Servomed, Stockholm, Sweden).[8] The TEWL value expressed as $g/m^2/h$ was registered on a pen recorder until a stable level was reached. Δ-TEWL is defined as the value for the test area minus the value for the control area recorded on the same occasion; it is thus an expression of the damage to the barrier layer within the lesion that increases the rate of water loss.

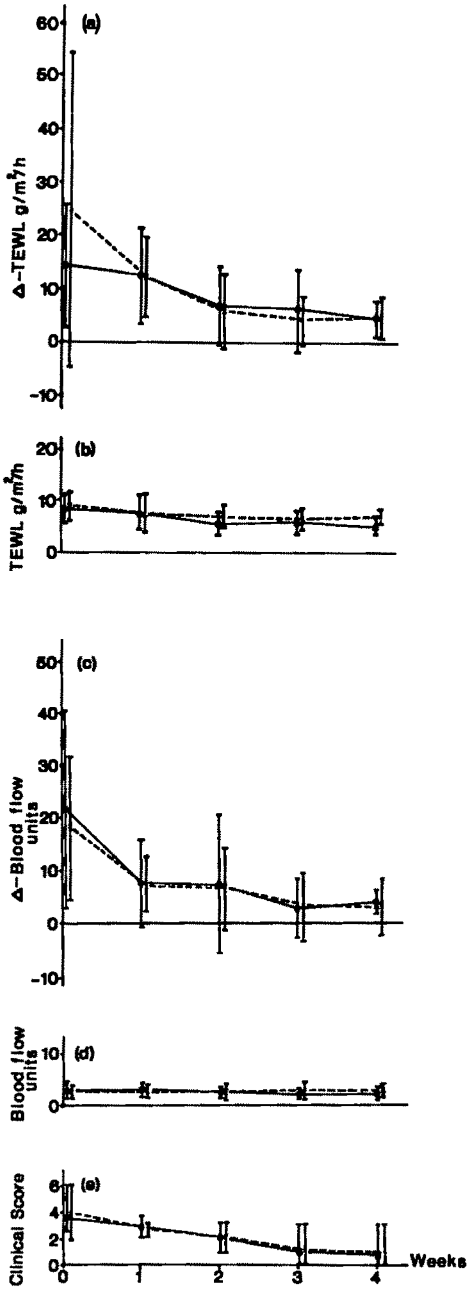

FIGURE 1. Transepidermal water loss (TEWL), laser Doppler flowmetry, and clinical scores in psoriatic lesions treated with the full Ingram regimen (————) and the modified Ingram regimen, i.e. tar substituted with oil emulsion (- - - -). (a) Mean difference between psoriasis lesion and control area (Δ-TEWL) ± SD. (b) Mean TEWL for control areas ± SD. (c) Mean difference in blood flow (Δ-blood flow) between psoriasis lesion and control area ± SD. (d) Mean blood flow for control areas ± SD. (e) Clinical score, mean and range. Week 0 = before start of treatment. Weeks 0 and 1, $n=11$; weeks 2 and 3, $n=10$; week 4, $n=8$.

The skin blood flow was measured with a prototype of the Periflux laser Doppler flowmeter (Perimed, Stockholm, Sweden).[9,10] The output signal of this instrument is linearly related to the product of the number of blood cells and their average velocity in the volume of tissue under study. The results are expressed in relative dimensionless units. The signal was registered on a pen-recorder until a stable level was reached. Results are expressed as the difference between the value for the test area and that for the control area recorded on the same occasion.

Statistical analysis

Student's *t*-test was used to compare results from the two test areas.

RESULTS

From the results of the clinical assessments (Fig. 1e) it can be seen that a process of successive healing took place during the first 3 weeks, after which most lesions had almost healed or showed only slight erythema. There was no difference in the rate of healing between the two test areas. The same conclusion was drawn from the photographic assessment, although with greater scatter. The Δ-TEWL values decreased with clinical healing, and were only slightly raised after 3 weeks of treatment. The results are given in Figure 1a. There was no significant difference between the two test areas. The large discrepancy between the two areas at week 0 is explained by an unusually high TEWL value for the lesion selected for oil emulsion in one patient. The TEWL values for the control areas varied very little during the study period (Fig. 1b).

The fall in blood flow was comparable to the fall in TEWL, and the blood flow values were only slightly greater than those for the control areas after 3 weeks of treatment. The values for the two test areas did not differ significantly. Changes in blood flow are shown in Figure 1c, and Figure 1d shows that the blood flow in control areas remained stable. The normalization of TEWL and blood flow closely parallelled the process of healing.

The greater scatter round the means of the Δ-TEWL and flowmetry values in the early part of the study period reflects the inter-individual variation in the initial state of the lesions.

DISCUSSION

The Ingram regimen is widely known to produce quick results in the treatment of psoriasis. In the present study the lesions had cleared or almost cleared within 3–4 weeks of treatment.

In a psoriasis plaque, TEWL is increased owing to damaged epidermal barrier function, and diminishes successively as healing proceeds. TEWL in unaffected skin of psoriasis patients does not differ from that in normal individuals.[4,11] The present study confirms that the initially high TEWL value in a psoriatic plaque returns towards normal at a rate parallelling the rate of healing.

In estimating the extent to which the TEWL value represents true transepidermal water loss the contribution of sweating must be taken into account. To minimize such interference the patient was allowed to relax in a quiet room at a comfortable temperature before the readings were taken. A bout of sweating is immediately reflected in a sudden rise in the TEWL value. The small fluctuations in TEWL recorded in the control areas indicate a minimal effect of environmental factors, such as changes in relative humidity and temperature, or of variations in hydration of the stratum corneum owing to bathing or the previous use of emollients. As an extra safeguard Δ-TEWL values were used to minimize any such influences.

A thick layer of scales could result in under-estimation of the TEWL value,[11] and purely desquamative treatment would thus give raised TEWL values. This seems to have occurred in a few instances in the present series after the first week of treatment, and could explain why the clinical score nevertheless showed improvement. However, the discrepancy was too small to affect the overall reduction in the mean value. The pathological stratum corneum of psoriasis lesions has a defective water binding capacity and thus a lowered water content.[12] This does not directly influence the TEWL value, however, because our method records only the rate of passage of water through the skin and not the actual water content.

Using the epicutaneous ^{133}Xe wash-out technique Klemp and Staberg found the cutaneous blood flow in psoriasis lesions to be considerably higher than that in normal skin.[13] In patients with widespread psoriasis they also recorded a higher than normal blood flow in unaffected skin. However, in patients with 'minimal' lesions of stable, chronic psoriasis the cutaneous and subcutaneous blood flow in unaffected skin did not differ from that in the skin of normal individuals.[14]

The Periflux laser Doppler flowmeter has been used previously to monitor skin blood flow in psoriasis and the effect upon this of Goeckerman or beech tar treatments.[6,7] A similar decrease with healing was reported to that found in the present study. No difference was found between unaffected skin and the skin of normal individuals.[7]

In the present study we recorded higher values for blood flow in psoriatic lesions than in unaffected skin. We also found that the blood flow, like the TEWL values, successively returned to normal with healing. Although the rate of blood flow seems to reflect closely the activity of the psoriatic process it could be argued that other factors also might influence the values. The laser Doppler technique records blood flow down to a depth of approximately 1 mm.[10] The process of healing will influence the degree of acanthosis and also the quantity of scales which are produced and which accumulate on the surface. It, therefore, follows that different strata of blood vessels could contribute successively to the signal, and this could mask the purely inflammatory component of the flow. As with TEWL values, blood flow was sometimes increased after the first week of treatment. This may have been the result of thinning of the scaly layer leading to an increase in the contribution to the signal from deeper and larger vessels. The same phenomenon was observed by Klemp and Staberg.[7] Cyclical and transient variations in blood flow may also contribute to fluctuations in recorded values; and owing to variations in the distribution of vessels the positioning of the probe must be taken into account.[15]

Like other workers,[16] we have found previously that laser Doppler signals can be used to measure UVB-induced erythema. We must stress explicitly, however, that this method only expresses the dynamics of blood flow, and is not generally applicable for measurement of the intensity of erythema. In the present study we occasionally recorded an increase in the laser Doppler signal in the control area, which could have been due to UVB exposure or to a toxic reaction following contamination of the skin with dithranol. On the whole, the control areas showed very stable blood flow throughout the study, indicating that UVB had been given at a suberythemogenic dose. On assessing the effect of treatment clinically and with the aid of evaporimetry and laser Doppler flowmetry, we were unable to detect any difference in the rate of healing of psoriasis treated with the Ingram regimen when the coal tar was replaced by oil emulsion. We conclude that coal tar extract has no advantage over oil emulsion in the Ingram regimen, but we cannot exclude possible benefits of coal tar in other treatment combinations. Coal tar baths create photosensitization, possibly with a peak sensitivity in the 330 nm region[17]. Suberythemogenic doses of UVB combined with tar oil are apparently as effective as erythemogenic UVB alone, and would thus have a UVB-saving effect.[18,19] However, a recent study has shown that this combination reduces the UVB dose to a mere 10% of the UVB dose in combination with the vehicle only.[20]

ACKNOWLEDGMENT

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A study of the role of house dust mite in atopic dermatitis

P.G.NORRIS, O.SCHOFIELD AND R.D.R.CAMP

Institute of Dermatology, St Thomas' Hospital, United Medical and Dental Schools, London, U.K.

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SUMMARY

Subjects with positive skin-prick tests to house dust mite (HDM) solution, including those with and without atopic dermatitis, participated in a double-blind, controlled study of the role of HDM exposure in the pathogenesis of atopic dermatitis. HDM solution and diluent control were applied daily to mildly eczematous or clinically uninvolved skin of the antecubital or popliteal fossae, without prior abrasion, for 5 days. Responses were assessed by a clinical grading system and by measurement of area of dermatitis; pruritus was recorded on visual analogue scales. The clinical grading system showed that marked or moderate delayed local reactions developed in one third of patients with atopic dermatitis in response to HDM application to both mildly eczematous and clinically uninvolved skin. Relative to control sites, significant increases in area of dermatitis and degree of pruritus were also recorded in response to HDM application to mildly eczematous sites. Application of HDM solution to normal, unabraded skin of prick test positive subjects without a history of dermatitis, produced pruritus and immediate urticarial responses which were not seen at control sites, findings which demonstrate that HDM antigen may be rapidly absorbed in normal skin. Application of vehicle or antigen solution to which subjects were negative on prick testing, produced no significant local reactions. This study provides objective evidence for a role for cutaneous HDM exposure in the pathogenesis of atopic dermatitis.

Patients with atopic dermatitis characteristically demonstrate positive skin-prick tests to multiple inhalant allergens,[1] including particularly the ubiquitous house dust mite (HDM) antigen, which elicits reactions in 90% of patients.[2] However, the pathogenetic role of HDM in atopic dermatitis remains controversial. Evidence favouring a role for this antigen includes the report that serum concentrations of HDM-specific IgE mirror disease severity[3] and the demonstration of percutaneous entry of HDM antigen.[4] Furthermore, uncontrolled studies have suggested improvement in atopic dermatitis following careful HDM avoidance regimens.[5–7] Eczematous responses to cutaneous challenge with HDM have also been demonstrated in patients with atopic dermatitis after abrasion[8] or scratching[4] of the test site, and after application of high antigen concentrations (500-fold that of a standard prick test

Correspondence: Dr P. Norris, Photobiology Unit, 11th Floor, North Wing, St Thomas' Hospital, London SE1 7EH, U.K.

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solution) to clinically normal skin followed by occlusion for 48 h.[9] Nevertheless, direct controlled evidence that HDM exposure exacerbates atopic dermatitis is lacking.

We report here a double-blind, controlled study of repeated cutaneous challenge with HDM antigen in skin-prick test positive subjects with and without atopic dermatitis.

METHODS

Subjects

Fifty two subjects took part in the study. All subjects gave informed consent and ethical committee approval was obtained. Details of subject groups are given in Table 1. All subjects in Groups 1, 2 and 5 were in-patients with atopic dermatitis, as defined by Hanifin and Rajka.[10] Subjects in Group 3 were atopic, but gave no history of atopic dermatitis, eight having had allergic rhinitis and two asthma, and were recruited from amongst hospital staff. Subjects in Group 4 were in-patients with negative skin-prick tests to HDM, and had endogenous dermatitis, including discoid eczema (two subjects), seborrhoeic dermatitis (two subjects) and diagnostically unclassified eczema (two subjects). No subjects had received oral antihistamine therapy during the 7 days prior to skin-prick testing.

Test solutions

The test solutions were kindly donated by Bencard, Brentford, U.K., and consisted of standard diagnostic preparations of *Dermatophagoides pteronyssinus* (code no. 2801), horse hair (HH; code no. 3203), and diluent containing 50% glycerol (v/v), 0·5% phenol (w/v), 6% sodium chloride (w/v), in water. These solutions were used both for prick testing and topical challenge.

HDM challenge

The subjects in Groups 1, 2, 4 and 5 had undergone in-patient treatment with topical corticosteroids and emollients for a variable period immediately before topical challenge, but during the 5-day challenge period all topical therapy including emollients was withheld from the

TABLE 1. Details of subjects who took part in the study

Group*	No. of subjects	Mean age years (range)	Skin prick test**		Test site	Challenge**
			DP	HH		
1 AD	20	24 (16–40)	Positive	ND†	Mild eczema	DP
2 AD	10	28 (19–65)	Positive	ND	Uninvolved	DP
3 Atopic	12	27·5(23–35)	Positive	ND	Normal	DP
4 ED	6	60 (44–76)	Negative	ND	Mild eczema	DP
5 AD	4	20·5(19–24)	ND	Negative	Mild eczema	HH

* AD: atopic dermatitis. Atopic: no history of atopic dermatitis. ED: endogenous dermatitis

** DP: *Dermatophagoides pteronyssinus*. HH: Horse hair

† ND: not determined

test sites. In Groups 1, 2 and 4 *D. pteronyssinus* prick-test solution (1 ml) was applied daily for 5 days to a 10 × 10 cm area on the antecubital or popliteal fossa and allowed to dry. The diluent control was applied in an identical fashion to the opposite limb. Following each application the test site was dressed with a light crepe bandage, sufficient to cover the test area without preventing scratching. No prior abrasion was performed. Subjects in Group 5 were similarly challenged with HH solution and diluent. Subjects in Group 3 were challenged with *D. pteronyssinus* solution and diluent on the antecubital fossae, as described above. In each subject in this group *D. pteronyssinus* solution (1 ml) was also applied to a 10 × 10 cm site on the upper back.

Assessment of response

Under double-blind conditions, an independent observer assessed the test sites for erythema, papules and excoriation using a grading system for each sign (absent = 0; mild = 1; moderate = 2; marked = 3) before the first application and 24h after the 5th application. The overall increase in score was used to estimate the degree of local deterioration (1 to 2 = mild; 3 to 4 = moderate; 5 or more = marked). Increase in area of dermatitis within the test site was measured double-blind by an independent observer, by comparison of the areas of tracings taken before the first application and 24 h after the 5th application. Areas were calculated using a digitizing tablet (Graphtek Corporation, Tokyo, Japan). Each day, all subjects rated the degree of pruritus over the preceding 24 h on a 10 cm visual analogue scale and the mean daily scores (expressed as distance from the origin in cm after subtraction of pre-challenge values) over the 5-day study period were calculated.

RESULTS

When challenged with *D. pteronyssinus* on mildly involved skin, one third of atopic dermatitis patients with positive skin-prick tests to HDM (Group 1) developed marked or moderate local deterioration after 5 days, as determined by the grading system (Table 2). This was associated with a significant increase in mean area of dermatitis (Table 3, and pruritus (Table 4) at antigen treated sites. An increase in pruritus of greater than 20% above that at control sites occurred in

TABLE 2. Deterioration as assessed by grading of erythema, papules and excoriation at test sites before and after 5-day study period

	Degree of deterioration (no. of subjects per group)**							
	HDM challenge				Control challenge			
Group*	Marked	Moderate	Mild	Unchanged	Marked	Moderate	Mild	Unchanged
1 AD	6	1	4	9	0	0	3	17
2 AD	1	2	1	6	0	0	1	9
3 Atopic	0	0	0	12	0	0	0	12
4 ED	0	0	1	5	0	0	1	5

* AD: atopic dermatitis. Atopic: no history of atopic dermatitis. ED: endogenous dermatitis.
** Deterioration determined by calculating overall increase in score after 5 day study period (see text).

TABLE 3. Increase in area of dermatitis at test site over study period

Group*	Increase in area (cm²) (mean ± SEM) HDM challenge	Control challenge	*P***
1 AD	7·9±2·4	0·1±0·6	<0·01
2 AD	1·9±1·2	0·2±0·2	NS
3 Atopic	0	0	NS
4 ED	−4·3±4·1	−6·8±6·8	NS

* AD: atopic dermatitis. Atopic: no history of atopic dermatitis. ED: endogenous dermatitis

** Paired *t*-test. NS: Not significant

13 out of 20 patients. Of 10 atopic dermatitis patients challenged on clinically uninvolved skin (Group 2), one third showed marked or moderate local deterioration after 5 days as determined by the grading system (Table 2). There was, however, less overall increase in area of dermatitis (Table 3) and pruritus (Table 4) than in Group 1 and the changes were not statistically significant.

When challenged with *D. pteronyssinus* on normal antecubital fossa skin, HDM prick-test positive subjects without atopic dermatitis (Group 3) did not deteriorate as determined by the grading system (Table 2), nor did they develop any measurable dermatitis (Table 3). However, nine out of 12 of these subjects showed local erythema and scattered, pruritic, urticarial papules beginning within 10 min of allergen application, and lasting up to 6 h. These were not seen following control applications. Thus, a significant increase in mean daily pruritus scores was recorded (Table 4). In this group, the mean pruritus score (± SEM) following HDM challenge on the antecubital fossa (2·9 ± 0·7 cm) was significantly greater than that following challenge on the upper back (1·3 ± 0·4 cm) ($P<0{\cdot}01$; Wilcoxon rank sum test; $n=12$). This assessment was not carried out under double-blind conditions.

TABLE 4. Mean daily increase in pruritus scores recorded on visual analogue scales during the study

Group*	Increase in pruritus score (cm) (mean ± SEM) HDM challenge	Control challenge	*P***
1 AD	1·4±0·4	0 2±0·2	<0·01
2 AD	1·0±0·4	0·3±0·2	NS
3 Atopic	2·9±0·7	0·2±0·4	<0·01
4 ED	0·1±0·2	0·1±0·2	NS

* AD: atopic dermatitis. Atopic: no history of atopic dermatitis. ED: endogenous dermatitis

** Wilcoxon's rank sum test. NS: Not significant

In-patients with eczema failed to show significant responses following repeated challenge with allergens (HDM or HH) to which they were skin-prick test negative (Groups 4 and 5).

DISCUSSION

In this study, repeated topical challenge of skin-prick test positive atopic dermatitis patients with HDM antigen caused significant delayed local reactions in one third of cases. This occurred in mildly eczematous test sites and also, to a lesser extent, in areas which were initially clinically uninvolved.

Immediate erythematous and papular urticarial responses were also seen in the majority of prick test positive subjects without atopic dermatitis, following topical application of HDM antigen to normal, unabraded skin. These findings demonstrate conclusively that HDM antigen may be rapidly absorbed, even in entirely normal, unoccluded skin, in which acute inflammatory reactions were elicited within minutes of topical application of standard unconcentrated prick test solution. Whether the rate of absorption of the HDM antigen was influenced by the composition of the vehicle used is currently uncertain. The lack of response to control application of vehicle, or to application of antigen to which patients were skin-prick test negative, implies an immunological rather than a non-specific irritant reaction to HDM in the responsive subjects. Possible explanations for the observed difference in immediate HDM-induced pruritus between test sites on the antecubital fossa and upper back, include regional variation in percutaneous absorption,[11] itch points[12] or mediators of pruritus, such as substance P.[13] This finding may provide a clue to the characteristic flexural distribution of atopic dermatitis in many patients.

Repeated application of HDM antigen to the skin without prior abrasion, but allowing scratching, more closely resembles the real situation than do patch-test models.[8] Estimates of the quantity of allergen applied in the present experiments, although approximate, allow comparison with likely environmental exposure; 1 ml *D. pteronyssinus* solution (Bencard) contains 60 μg HDM P1 antigen,[14] 5–100 μg being recoverable from 1 g crude house dust,[15] which in turn may be obtained by vacuum cleaning an area of mattress of up to 1 m^2 for 2 min.[16]

Although the present study provides objective evidence for a causal role for HDM in a percentage of patients with atopic dermatitis, the question remains as to why certain HDM responsive patients develop atopic dermatitis whereas others, who are prick test positive and develop acute reactions to open topical application of HDM (Group 3), do not. Further characterization of the reactions obtained following topical application of HDM antigen in these different subject groups may provide a fruitful avenue of reasearch into this question.

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Pustular ulcerative dermatosis of the scalp

W.K.JACYK

German Leprosy Relief Assocation, National Leprosy Training Centre, Wau, Sudan

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SUMMARY

Six young African patients are described with erosive pustular scalp lesions leading to extensive ulceration. No bacterial or fungal cause was found. Biopsies showed non-specific changes of atrophy with subacute or chronic inflammation. Four patients were malnourished and anaemic and three were infested with hookworm. The condition did not respond to antibiotics. Healing was obtained in one patient with systemic steroids. The relationship of this disorder to erosive pustular dermatosis of the scalp and to pyoderma gangrenosum of atypical distribution is discussed.

It is extremely unusual to encounter a chronic destructive pyoderma which is confined to the scalp, and which is not produced by any infecting organisms. A few cases of this kind have been described under the title of pyoderma gangrenosum,[1–3] but an exclusive localization to the scalp is admitted to be very rare.

Burton[4] and later Pye *et al.*[5] have described a distinctive pustular, erosive and scarring dermatosis of the scalp, which occurs in elderly white women. Other reports[6–8] have established the validity of this clinical picture.

I report here six cases of a condition with similar clinical features in young Africans living under conditions of social disadvantage.

CASE REPORTS

Five of the patients were male and one female. The clinical features are summarised in Table 1. Patients 1, 2 and 3 were all Dinkas from the Southern Sudan, and they were studied in 1982. Patients 4, 5 and 6 were seen during 1974–76 in Nigeria.

The course of the disease and the clinical appearance of the scalp lesions were similar in all the patients. Pustulation was present for a few weeks followed by extensive erosions and superficial ulceration. The pustulation fluctuated for no apparent reason. The vertex of the scalp was affected in all patients with a variable degree of spread to the adjacent areas. The base of the ulcerations was moist and beefy red, with good granulation and only a scanty exudate. There

Correspondence: Dr W.K.Jacyk, Kalafong Hospital, Department of Dermatology, P.O. Box X396, 0001 Pretoria, Republic of South Africa.

TABLE 1. Clinical details of the patients studied

	Patient					
	1	2	3	4	5	6
Age (years)	29	25	8	15	6	5
Sex	M	M	M	M	M	F
Ethnic group	Dinka	Dinka	Dinka	Hausa	Hausa	Hausa
Country of origin	Sudan	Sudan	Sudan	Nigeria	Nigeria	Nigeria
Age at onset (years)	12	13	7	9	6	?
Location of the scalp ulcerations	Vertex	Vertex upper occiput, parietal	Vertex	Vertex, upper occiput, upper parietal	Vetex, upper occiput, parietal	Vertex, occiput, parietal, zygomatic
Other findings	Undernourished Hb 10·5 g/dl Hookworm	Grossly cachectic Hb 8·8 g/dl Hookworm	Undernourished Hb 8·6 g/dl Hookworm	Undernourished Hb 11·2 g/dl	—	—

was no tendency to crusting. The edge of the lesions was thin and not undermined. The pustules were of different sizes, from pinpoints to 'pus lakes', reaching 5 mm in diameter.

Scalp lesions in Patients 1, 2, 5 and 6 are shown in Figure 1. Regional peripheral lymph nodes were not enlarged. Repeated cultures from the pustules in Patients 1, 2 and 3 grew only diphtheroids, and in one case *Staphylococcus epidermidis*. Biopsies from two patients (Patients 1 and 2) taken from the edge of the ulceration showed atrophic and flattened epidermis and a diffuse infiltrate in the upper dermis consisting of numerous plasma cells, lymphocytes and polymorphonuclear leukocytes. Hair follicles were absent. The number of blood vessels

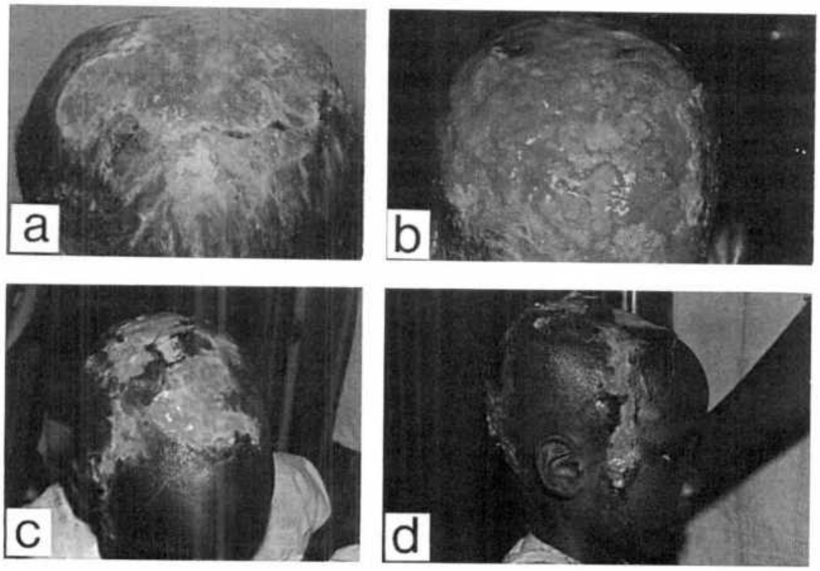

FIGURE 1. Scalp lesions. (a) Patient 1; (b) Patient 2; (c) Patient 5; (d) Patient 6.

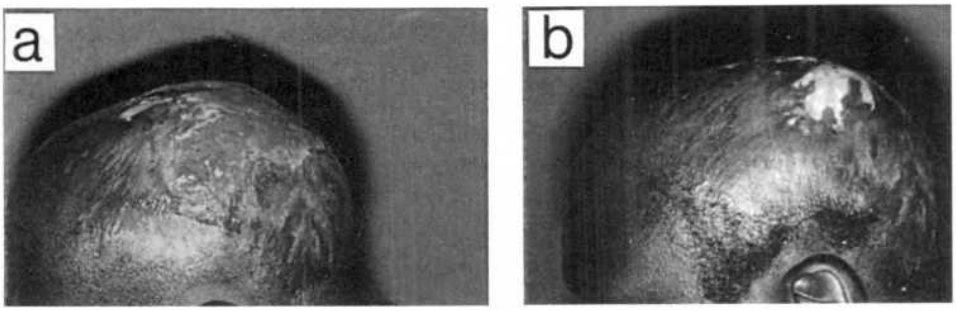

FIGURE 2. Patient 1. (a) Before treatment and (b) after 16 weeks of treatment with oral dexamethasone.

appeared to be increased. Four patients were evidently undernourished, and one of these (Patient 2) was grossly cachectic. None of the patients gave a history or showed clinical signs of inflammatory bowel disease. Radiological investigations of the alimentary tract were not performed. All four patients (Patients 1, 2, 3 and 4) in whom routine laboratory investigations were performed (FBC, urinalysis, VDRL, serum transaminases and stool examination for parasites) showed hypochromic anaemia and peripheral eosinophilia. In three of these patients the eosinophilia could be attributed to hookworm infestation. Chest X-rays in Patients 1, 2 and 3 showed no pathology.

Treatment

Three patients (Patients 1, 2 and 3) were hospitalized. In Patient 1, complete healing of the scalp lesions was achieved with oral dexamethasone. This patient's lesions before and after treatment are shown in Figure 2. The initial dexamethasone dose of 7 mg/day was slowly reduced over a period of 16 weeks. Previous treatment with oral erythromycin 1·0 g/day and clofazimine (Lamprene®) 200 mg/day had not brought about any significant improvement.

Patients 2 and 3 received Lamprene 200 mg/day, oral erythromycin 1·0 g/day and a topical steroid preparation (Nerisone C®) for 4 weeks. No improvement was observed. All three patients were given, in addition, oral iron preparations and a course of Bephenium for hookworm.

The Nigerian patients (Patients 4, 5 and 6) had been treated solely with oral and topical antibiotics. Follow-up in these patients was too short and incomplete to warrant any conclusions.

DISCUSSION

No condition identical to that described in the present series of patients has been reported to my knowledge. Peachey[1] and Powell and Perry[2] observed cases of pyoderma gangrenosum of the scalp. Their patients, both girls, in whom the onset of the cutaneous ulceration was at the age of 10 and 12 years, respectively, also showed lesions on the extremities or the trunk. They also suffered from inflammatory bowel disease. Scalp lesions were also present in two of the six patients with pyoderma gangrenosum involving the head and neck reported by Snyder.[3] Both these patients, men aged 40 and 33 respectively, had inflammatory bowel disease and one of them also had ulceration on the leg. Although pustular elements were evident in Snyder's cases,[3] the ensuing ulceration had a cribriform and undermined border, and crusting. These features were not observed in our patients.

In patients reported as suffering from malignant pyoderma,[9–13] the head and neck were affected, with a predilection for the periauricular region. The scalp, however, was spared. Ulcerative lesions in malignant pyoderma were preceded more often by multiple inflammatory nodules, abscesses and draining sinus tracts than by surface pustules. The existence of malignant pyoderma as a separate entity is controversial and in a recent paper Wernikoff *et al.*[14] conclude that pyoderma gangrenosum and malignant pyoderma are probably identical disorders.

Verbov[15] described a case of erosive candidiasis of the scalp in an elderly woman. The scalp in this case showed an erythematous, erosive, scabbed and pustular eruption and *Candida albicans* grew from scalp swabs. There was also oral thrush, and the nails were infected by the same pathogen. The scalp lesions in our patients with pustulation leading to eroded areas and healing with atrophic scarring and alopecia are typical of the clinical behaviour of erosive pustular dermatosis. There are, however, certain distinct features: the young age of the patients, the occurrence in both sexes, but with a predominance in males, the greater extent of the lesions, and a striking absence of crusting. Repeated cultures in three patients revealed no significant bacterial pathogen, nor *Candida.*

The histology was non-specific. Other simple investigations, which were all that could be carried out due to unavailability of more sophisticated facilities, were essentially negative. Hypochromic anaemia, which was found in all four patients in whom a full blood count was performed, arose most probably from hookworm infestation and malnourishment. The treatment of the condition was difficult. Topical and systemic antibiotics as well as clofazimine and topical steroids had little effect. Systemic steroids resulted in marked improvement in the only patient who showed good compliance. The patients described here may well represent a variant of non-infective erosive pustular dermatosis of the scalp occurring particularly in children and young adults; nutritional factors, iron deficiency and parasitic infestations may contribute to the occurrence of the dermatosis.

I suggest the name pustular ulcerative dermatosis of the scalp to indicate that the severity of the condition exceeds a simple erosive state.

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Two unusual cases of orf following trauma to the scalp

J.REES, AND JANET M.MARKS

University Department of Dermatology, Newcastle upon Tyne, U.K.

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SUMMARY

Two cases of orf of the scalp following trauma produced by an inanimate object are described.

Orf, a poxvirus, characteristically produces lesions on the hands from direct inoculation of infected material.[1] We report here two cases of virologically proven orf of the scalp following scalp trauma.

CASE REPORTS

Case 1

A 59-year-old mechanic presented to his general practitioner with a ten day history of a swelling of his scalp overlying the right parietal bone (Fig. 1). Eight days earlier he had knocked his head on a steel beam in a farm building, resulting in a small skin abrasion. On examination there was a 2 × 3 cm pustular nodule at the site of the previous injury and a 2 cm diameter palpable lymph node in the posterior triangle of the neck. General examination and routine blood tests were unremarkable. The clinical differential diagnosis lay between pyogenic granuloma, malignancy and orf. The lesion was curetted and electron microscopy showed particles with the typical morphology of orf. He received no specific therapy and the lesion subsided over the next 4 weeks.

Case 2

A 61-year-old shepherd presented to a general surgeon with a 10-day history of a lesion on his scalp overlying the left parietal bone (Fig. 2). Three weeks earlier he had knocked his head on a tractor door, sustaining bruises. On examination there was a 3 cm diameter haemorrhagic pustule on the scalp and tender nodes in the posterior triangle of the neck. General examination was normal apart from a mild pyrexia. The lesion orginally was thought to be an infected pyogenic granuloma by the surgeons. However, the dermatologists' differential diagnosis in this

Correspondence: Dr J.M.Marks, University Department of Dermatology, Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP, U.K.

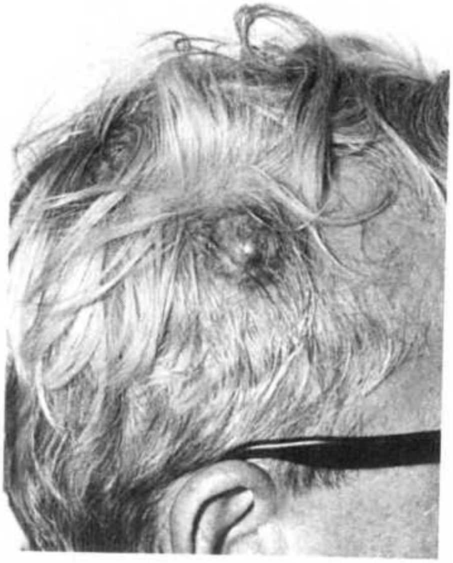

FIGURE 1. Clinical appearance of the lesion in Case 1.

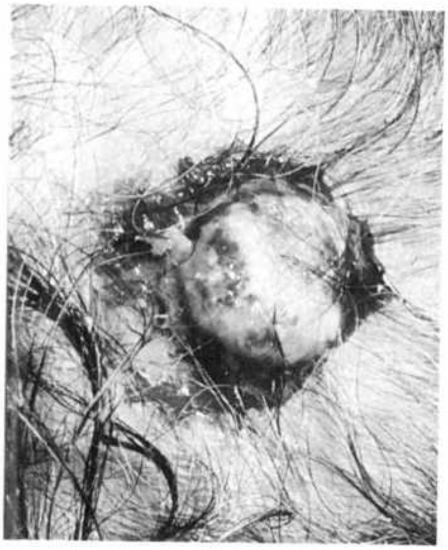

FIGURE 2. Clinical appearance of the lesion in Case 2.

case also, was pyogenic granuloma, orf or a malignant tumour. Electron microscopy showed particles with the characteristic appearance of orf. The lesions subsided over the next month.

DISCUSSION

Orf has a worldwide distribution and is common in sheep, particularly young lambs. Infection in sheep is usually transmitted from animal to animal, but the disease may also be contracted from virus persisting in the pastures.[2] Infections in man are usually reported on the hands and forearms, and occasionally on the face or head.[3] They are thought to be the result of direct inoculation of infected material from animals, although cases without a clear history of this have been reported. In both our cases the unusual site led to initial uncertainty in the diagnosis. The clear history of trauma at the site of the subsequent lesion is suggestive of viral inoculation from an inanimate object.

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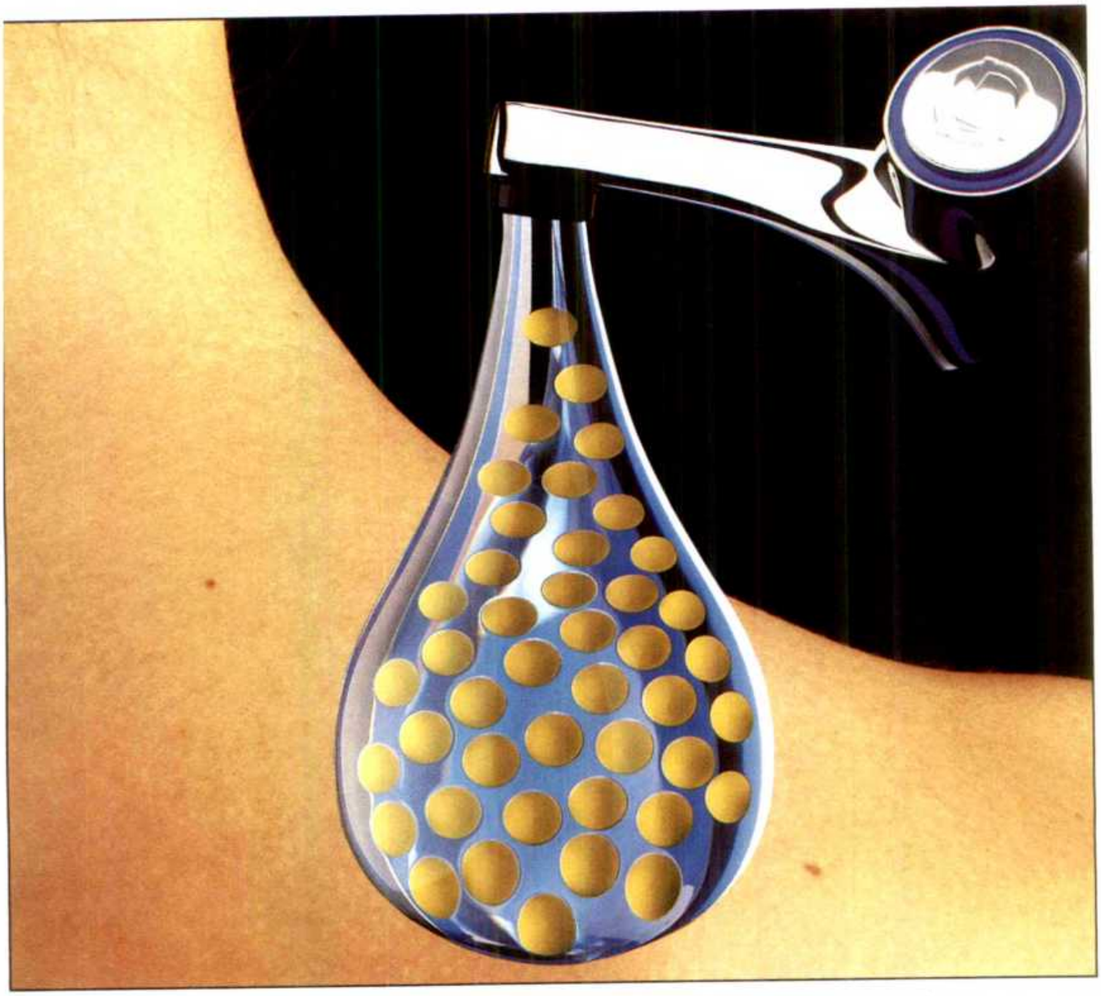

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Correspondence

Oral acylovir in herpes simplex virus associated erythema multiforme

MADAM, I read with interest the article by Molin (*British Journal of Dermatology* 1987 **116**, 109). The association of herpes simplex infection and erythema multiforme is an increasingly frequent event in young people. The results reported are very encouraging. I should like to enquire whether the author has any evidence of the emergence of resistant strains of the virus with prolonged therapy. Such a possibility has been reported recently.[1]

Clinica Dermatologica,
Università di Ferrara,
44100 Ferrara, Italy

RENATA STRUMIA

REFERENCE

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Reply

MADAM, Thank you for giving me the opportunity to reply to Dr Strumia's letter regarding the risk of decreased sensitivity of herpes simplex virus to acyclovir. I agree that this is a highly relevant question. We have tested the sensitivity of herpes virus isolated before and after long-term daily oral acyclovir treatment (6–21 months) in eight patients with frequently recurrent genital herpes, and found no evidence of decreased sensitivity.[1] This aspect is also under study in another long-term treatment trial in which no case of decreased sensitivity has been found. Svennerholm *et al.*[2] also found no evidence of decreased or altered sensitivity to acyclovir in herpes virus strains isolated during recurrent episodes of genital herpes.

Department of Dermatology,
University Hospital.
5-581 85 Linköping, Sweden

L. MOLIN

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Treatment of atopic eczema with evening primrose oil

MADAM, The paper by Schalin-Karrila *et al.* (*British Journal of Dermatology* 1987, **117**, 11) which reports results of a double-blind trial contains the following statements: 'The patients receiving evening primrose oil (EPO) showed a significantly greater reduction in inflammation than placebo.' 'A statistically significant difference in degree of inflammation was found between the two groups.' 'In a between groups analysis a significant difference was found at 12 weeks for inflammation.'

Whilst these statements are not untrue in a literal sense, they convey a misleading impression of what the trial actually showed. The following were not made clear by the authors: (1) EPO conferred little or no significant clinical benefit in this study, compared with placebo. (2) There is no significant difference between the two treatment groups. Had the authors stated confidence intervals for their data this would have been numerically obvious. (3) No direct comparison between the two groups at particular time intervals showed a significant difference. (4) The authors claim that a significant difference was found at 12 weeks for inflammation is puzzling. In fact, as an inspection of Figure 1 in their paper shows, scores for inflammation at 12 weeks were almost identical in the two groups.

All that the authors have actually found in this study is that although both placebo and EPO groups improved, the EPO group improved marginally faster than the control. Whether this represents a clinically useful difference is a moot point, to say the least.

St Johns Hospital for Diseases of the Skin,
Lisle Street, Leicester Square,
London WC2H 7BJ, U.K.

M.W. Greaves
Margaret F. Corbett

Reply

Madam, We thank Professor Greaves and Dr Corbett for their comments. However, we think these are misleading and in some respects untrue, in part because of our oversight in not correcting Figure 1 after the

Table 1. Analysis of effect of treatment with EPO (Efamol) and placebo in atopic eczema

						Effect of treatment Wilcoxon signed ranks test			Difference between treatments Wilcoxon Sum of Ranks Test	
	Weeks	Treatments	*n*	Baseline median (week 0)	Median improvement	R	*n*	$2P$	W	$2P$
Overall severity	0–6	Efamol	11	49	7	16	11	0·134	69	0.806
		Placebo	7	39	5	9	7	0·372		
	0–12	Efamol	10	49	17	5	10	0·018*	71	0·463
		Placebo	7	39	11	5	7	0·182		
Area involved (%)	0–6	Efamol	13	40	10	4	10	0·021*	163	0·149
		Placebo	11	30	0	4	5	0·343		
	0–12	Efamol	13	40	12·5	0	11	0·000***	170	0·056
		Placebo	11	30	0	2	6	0·090		
Overall response	0–6	Efamol	13	—	1	0	11	0·000***	127	0·583
		Placebo	10	—	1	4	9	0·020*		
	0–12	Efamol	13	—	2	0	11	0·000***	116	0·173
		Placebo	11	—	1	6	10	0·034*		
Inflammation	0–6	Efamol	14	2	0·5	5	8	0·073	147	0·807
		Placebo	11	2	0	4	6	0·234		
	0–12	Efamol	13	2	1	0	11	0·000***	150	0·433
		Placebo	11	2	1	0	7	0·016*		
Dryness	0–6	Efamol	14	2	0·5	0	7	0·016*	153	0·562
		Placebo	11	2	0	4	6	0·234		
	0–12	Efamol	13	2	1	0	7	0·016**	151	0·403
		Placebo	11	2	0	7	7	0·356		
Itch	0–6	Efamol	14	1	0·5	0	7	0·020*	171	0·100
		Placebo	11	1	0	11	7	0·812		
	0–12	Efamol	13	1	1	0	8	0·007**	174	0·036*
		Placebo	11	1	0	11	7	0·818		

Overall severity measured on a visual analogue scale 0–100
Overall response: measured on a 6 point scale -2 = Much worse to 3 = Cured
Inflammation, Dryness and Itch: measured on a 4 point scale, 0 = None to 3 = Severe
* $2P \leqslant 0{\cdot}05$
** $2P \leqslant 0{\cdot}01$
*** $2P \leqslant 0{\cdot}001$

paper had been revised in the light of comments by a referee. We also failed to pick up errors in line 6 of the summary and in line 40 on page 13. At both points the printed 'inflammation' should have been 'itch', which answers point 4.

During the review process a referee specifically requested that non-parametric statistics should be used. The full details are shown in Table 1. It is on this table that the text was based and we should have revised Figure 1 in accordance with it.

The main point being made by Professor Greaves and Dr Corbett seems to be that the differences between the Efamol, evening primrose oil (EPO) and placebo groups are marginal and of doubtful clinical importance. We take a different view on the following grounds: (1) For all six clinical parameters assessed, EPO produced a significant improvement over baseline; for four of the six the P value was 0·01 or less. Placebo produced significant improvements over baseline in only two of the six parameters. (2) For every clinical parameter, except inflammation, the difference between the response to EPO and to placebo was in favour of EPO. This difference reached significance in the case of itch and came close to it for the area of skin involved. (3) Use of topical steroids in the EPO group was only 30% of that in the placebo group. This difference between the groups was significant. Therefore, the clinical improvements on EPO were achieved in spite of sharply reduced steroid use. (4) For five of the six clinical parameters, the differences between the EPO and placebo groups were greater at 12 weeks than at 6 weeks. It is, therefore, untrue to say that the differences between the two groups could simply be accounted for by more rapid improvement in the EPO group.

The consistent responses of all parameters to EPO, the consistent differences (especially with regard to ich) between responses to EPO and placebo, the reduction in topical steroid use in the EPO group to less than a third of that in the placebo group and the increasing differences between EPO and placebo as the trial progressed, all indicate that EPO was having a clinically important as well as a statistically significant effect. Finally, we would point out that the biochemical rationale for using EPO has recently been strengthened by an independent study which indicated, on the basis of measurements in umbilical cord blood and in atopic children, that the metabolism of linoleic acid is indeed impaired in atopic eczema.[1]

Department of Dermatology,
University of Turku,
SF-20520 Turku 52,
Finland
*Efamol Ltd,
Woodbridge Meadows,
Guildford, Surrey GU1 1BA, U.K.

C.T. JANSEN
P. MORSE*

REFERENCE

1 Strannegard I-L, Svennerholm L, Strannegard O. Essential fatty acids in serum lecithin of children with atopic dermatitis and in umbilical cord serum of infants with high or low IgE levels. *Int Arch Allergy Appl Immunol* 1987; **82:** 422.

Control of plantar blisters in pachyonychia congenita with topical aluminium chloride

MADAM, The letter from Drs Soyuer and Candan (*British Journal of Dermatology*, 1987, **117**:264), concerning the disappointing results obtained with the use of etretinate to treat the palmoplantar keratoderma of pachyonychia congenita, has prompted us to report a simple, but quite effective, remedy for the pedal blistering frequently suffered by those with this condition. The blisters in pachyonychia congenita form within the stratum spinosum (personal observation) and are histologically indistinguishable from normal friction blisters. Hyperhidrosis, which is a usual feature of pachyonychia congenita, is known to increase the proclivity to blisters by influencing the coefficient of friction of the skin,[1] and this fact provided the rationale for the use of a topical antiperspirant in a 25-year-old female with the

Jadassohn-Lewandowsky variant of pachyonychia congenita. Since childhood the patient had developed painful blisters on the soles of her feet after minimal exercise, particularly during the summer months. However, within a few weeks of applying 20% aluminium chloride hexahydrate in alcoholic solution to her soles night and morning, she noticed a diminution of the hyperhidrosis concomitant with an appreciable reduction in the tendency to form blisters, which persisted with the continued use of the antiperspirant. Topical aluminium chloride has also been used successfully to control blistering in the Weber-Cockayne type of epidermolysis bullosa simplex,[2] and the simple expedient of reducing sweat production may ameliorate a distressing symptom of otherwise untreatable genodermatoses.

Department of Dermatology,
Guy's Hospital,
St Thomas Street, London SE1 9RT, U.K.

M.J. TIDMAN
R.S. WELLS

REFERENCES

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2 Tkach JR. Treatment of recurrent bullous eruption of the hands and feet (Weber-Cockayne disease) with topical aluminium chloride. *J Am Acad Dermatol* 1982; 6: 1095–96.

Mepacrine and pregnancy

MADAM, We wish to report the successful outcome of two consecutive pregnancies in a patient who took mepacrine 100 mg daily for the first 4 weeks of each gestation period. Mepacrine had been prescribed for the treatment of discoid lupus erythematosus and the patient advised against becoming pregnant whilst taking the drug. On both occasions she stopped taking mepacrine as soon as she suspected that she was pregnant.

Eighth nerve damage, posterior column defects, mental retardation, neonatal convulsions and retinal damage have all been reported in association with maternal ingestion of chloroquine during pregnancy.[1,2] However, there is little useful information regarding mepacrine, despite its extensive use in the treatment and prophylaxis of malaria. One animal study has shown an increased incidence of fetal death with high doses of mepacrine in rats.[3] There is also a case report of renal agenesis and hydrocephalus occurring in a human infant whose mother took mepacrine 100 mg daily for the first 2 weeks of pregnancy,[4] however, it is questionable whether these abnormalities were related to ingestion of the drug in this case as organogenesis takes place later in gestation and the fetus is thought to be more 'resistant' to such insults during the first few weeks of development.

Our patient's two children, now aged 3 years and 4 months, respectively are both normal. The older child had a brief systolic murmer noted at birth which subsequently became inaudible. Whilst we would not advise the use of mepacrine during pregnancy this information may be of use in the future should other patients inadvertently become pregnant whilst taking this drug.

University Department of Dermatology,
Royal Victoria Infirmary,
Newcastle upon Tyne NE1 4LP, U.K.

FRANCES HUMPHREYS
JANET M. MARKS

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2 Paufique L, Magnard P. Degenerescence retinienne chez deux enfants consecutive a un traitement antipaludeen chez la mere pendant la grossesse. *Bull Soc Ophthalmol* 1969, 69: 466—7.
3 Rothschild B, Levy G, Action de la quinacrine sur la gestation chez la rate. *C R Soc Biol* 1950, 144: 1350-1352.
4 Nishimura H, Tanimura T. Clinical aspects of the teratogenicity of drugs. *Exerpta Medica* 1976.

Fish oil for eczema

MADAM, Tilbury Fox would have been pleased to read that fish oil is beneficial for atopic eczema (Bjørneboe *et al. British Journal of Dermatology* 1987; 117: 463). In 1877 he wrote 'If eczema be present, cod liver oil must be given freely', and for prurigo simplex, 'No remedy is so good internally as cod liver oil'.[1]

Department of Dermatology,
Bristol Royal Infirmary,
Bristol BS2 8HW, U.K.

J.L.BURTON

REFERENCE

1 Fox T. *Atlas of Skin Diseases*. 1877; London: J.A.Churchill, p. 69.

Editor's note

On behalf of my illustrious predecessor, Professor T.McCall Anderson, I am happy to pre-empt Dr Burton's claim that Dr Tilbury Fox was the first to report on the value of cod liver oil in psoriasis in 1877. Professor McCall Anderson was both a dermatologist and Professor of Medicine at Glasgow University in the late 19th century. In his beautifully illustrated and extremely informative text on psoriasis and lepra, published in 1865 by Churchill, he states on page 42 that in the treatment of psoriasis cod liver oil is 'Next in efficacy to arsenic in the internal treatment of psoriasis, though applicable to a different class of cases'. A fine pale oil is recommended and the maximum dose is four tablespoonfuls per day. The following paragraph could well apply to our current patients concerning fish oil therapy: 'When it is said to disagree with the stomach it in reality agrees quite well, but is disagreeable to the taste. On no account should the patient be allowed to stop it for this slight reason'!

I am very grateful to Dr V. Dave of Manchester for drawing my attention to a reference which was actually to be found on my own personal bookshelf.

RONA M.MACKIE

British Journal of Dermatology (1988) **118**, 455–456

News and Notices

Forthcoming Cambridge Courses

Progress in Biological Sciences in Dermatology
The 7th 5-yearly Course will be held in Churchill College, Cambridge, 3–5 October 1988. This is aimed to update practising clinical dermatologists in some advances in basic sciences in the past 5 years.

Introductory Course on the Biology of the Skin
The annual Introductory Course on the Biology of the Skin, primarily for registrars and postgraduate students at an early stage of their training in dermatology, will be held as usual at Fitzwilliam College, Cambridge, 12–16 December 1988.

Further information may be obtained from: The Conference Secretary (BoS) Postgraduate Medical Centre, The Clinical School, Addenbrooke's Hospital, Hills Road, Cambridge CB2 2QQ, U.K. Tel: (0223) 212216.

Dermatopathology Foundation 15th Annual Course on Gross and Microscopic Pathology of the Skin

The 15th annual course on gross and microscopic pathology of the skin will be held in the Atlantis Casino Hotel, Atlantic City, New Jersey, U.S.A. from 13 to 17 June 1988. For further details contact: Dermatopathology Foundation, P.O. Box 377, Canton, MA 02021, U.S.A. Tel: 617-821-0648.

1st Mediterranean Symposium on Behçet's Disease

The first Mediterranean Symposium on Behçet's Disease, organized by the Turkish Rheumatism Society and the Turkish Society against Behçet's Disease, will be held in Instanbul, Turkey on 4 June 1988. The programme will include a workshop on vasculitis in Behçet's disease, lectures by invited speakers and free papers and posters. The symposium languages will be English, French and Turkish. For further information contact: Professor N. Dilşen, Istanbul Faculty of Medicine, Division of Rheumatology, Çapa, Topkapi, Istanbul, Turkey.

First Greek/Australian International Medical and Legal Conference

The first Greek/Australian International Medical and Legal Conference will be held in the Hotel Athenaeum Intercontinental, Athens, Greece, from 29 May to 3 June 1988. The theme of the conference is 'Bridging two cultures in law and medicine'. We all recognize the legacy of ancient Greece to medicine and its contribution to our legal system. The conference organizing committee has prepared a most exciting and stimulating professional programme with eminent speakers from both Greece and Australia. These include Sir Daryl Dawson, Sir Zelman Cowen, Mr Justice Phillips, Professor Graham Burrows, Mr Bill Moyle, Professor Koumantos and Professor Doxiadis. It is the aim of the organizing committee to make this the first of a series of bi-annual Greek/Australian international medical and legal conferences. The location of the opening session will be the ancient Herod Atticus Theatre on the Acropolis. The opening will be performed by the Minister for Culture in Greece, Mrs Melina Mecouri, and will be followed by the performance of a classical Greek play. Further details from: First Greek/Australian International Medical and Legal Conference, Athens Greece 1988 Ltd, c/o Secretariat, I.C.M.S. P.O. Box 29, Parkville, Victoria, Australia 3052.

Psoriasis Association Research Grants

The Psoriasis Association invites papers from researchers interested in applying for a grant, especially for work on the basic causes of psoriasis. Papers for consideration in respect of the 1988 awards should be lodged before 1 May 1988 with Mrs L. A. Henley, Secretary, Medical and Research Committee, The Psoriasis Association, 7 Milton Street, Northampton NN2 7JG U.K. Telephone: 0604 711129

International Epidermolysis Bullosa Symposium

The International Epidermolysis Bullosa Symposium will be held on 18–19 January, 1989, in London, U.K., immediately preceding the clinically orientated symposium on bullous disorders organised in association with the European Society for Dermatological Research. This meeting will be of interest to members of all the different disciplines involved in the management of epidermolysis bullosa sufferers. Invited speakers will lecture on advances in research and on wide-ranging clinical topics. There will be free communication sessions. Closing date for receipt of abstracts 30 June 1988. For further information and abstract forms contact Dr R. A. J. Eady, British Association of Dermatologists, 6 St. Andrews Place, Regents Park, London NW1 4LB. U.K.

Clinically Orientated Symposium on Bullous Disorders

A clinically orientated symposium on bullous disorders will be held on 20–21 January 1989 in London, UK, in association with the European Society for Dermatological Research. Invited speakers will lecture on topics covering the diagnosis and therapy of the blistering diseases and recent research advances. There will also be free communication sessions. Closing date for receipt of abstacts 30 June 1988. This meeting will be immediately preceded by the International Symposium on Epidermolysis Bullosa. For further information and abstract forms contact Dr R. A. J. Eady, British Association of Dermatology, 6 St. Andrews Place, Regents Park, London, NW1 4LB.

Stiefel Travelling Grants

Through the auspices of Stiefel Laboratories UK Ltd., the BAD has available a limited number of travelling grants to help registrars, senior registrars and consultant dermatologists, or those with similar academic status, working in dermatology, to attend international and national meetings abroad. Applicants must provide evidence of active participation in the meeting. For further information please contact: British Association of Dermatologists, 6 St Andrews Place, Regents Park, London NW1 4LB, U.K. Closing date for receipt of completed application forms is 30 June 1988.

Erratum

Sweat-gland necrosis after beta-adrenergic antagonist treatment in a patient with pheochromocytoma, Naeyaert et al., 1987; 117: 371. The running title of this paper should have read 'β-antagonist-induced sweat-gland necrosis', and in the third paragraph on page 372: 'the urinary excretion of noradrenalin was 11,782 μg/24 h (normal 100 μg/24 h).

British Journal of Dermatology (1988) **118**, 457–470.

Effects of topical corticosteroid therapy on Langerhans cell antigen presenting function in human skin

J. ASHWORTH, JULIE BOOKER AND S.M.BREATHNACH

Department of Medicine (Dermatology), Charing Cross and Westminster Medical School, Fulham Palace Road, London W6 8RF, U.K.

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SUMMARY

We have investigated the mechanisms by which topical corticosteroids modulate cutaneous immune reactions in man. Volunteers applied clobetasone butyrate 0·05% (Eumovate®; EV), betamethasone valerate 0·1% (Betnovate®; BV), clobetasol propionate 0·05% (Dermovate®; DV), and control vehicles twice daily to forearm skin for 7 days. Steroid therapy significantly decreased the number of HLA-DR/T6 (CD1a) positive Langerhans cells (LCs) per mm^2 in suction blister-derived epidermal sheets, expressed as a mean percentage of controls, as follows: EV 69·2%; BV 67·3%; DV 37·8%. LC antigen presenting capacity was determined in the allogeneic and autologous epidermal cell–lymphocyte reactions. The LC-dependent allostimulatory capacity of epidermal cells, expressed as a mean percentage of controls, was also significantly reduced by steroid therapy: EV 45·1%; BV 41·9%; DV 23·4%. Following therapy with clobetasol propionate 0·05%, the capacity of epidermal cells to present tetanus toxoid to, and to augment concanavalin A mediated lymphocyte stimulation of, autologous lymphocytes was reduced to 33·6% and 19·7% respectively of controls. Depression of epidermal cell allostimulatory capacity was not the result of a steroid-induced decrease in the production of epidermal cell-derived thymocyte activating factor (ETAF)/interleukin 1 by keratinocytes, since it could not be reversed by addition of exogenous interleukin 1. Indomethacin, added to block any potential prostaglandin synthesis during the culture period, did not restore the allostimulatory capacity of epidermal cells from steroid-treated sites. Addition of epidermal cells from DV-treated sites depressed the capacity of control epidermal cells to stimulate lymphocytes in the allogeneic epidermal–lymphocyte reaction. Our results demonstrate that the anti-inflammatory action of topical corticosteroids in man is associated not only with a reduction in the number of HLA-DR/T6 positive LCs, but also with a marked decrease in Langerhans cell-dependent T lymphocyte activation. The effects of the different steroids on both of these parameters correlated with their potency as determined in the standard occlusive vasoconstrictor assay.

Correspondence: Dr J. Ashworth.

Topical corticosteroids are widely used for the treatment of inflammatory skin disorders, and inhibit not only the elicitation phase, but also the induction phase, of allergic contact dermatitis reactions.[1,2] The immunosuppressive actions of topical corticosteroids are incompletely understood, but are thought to be at least in part the result of effects on the skin-associated immune system, comprising bone marrow-derived, dendritic antigen-presenting epidermal Langerhans cells (LCs), and keratinocytes which secrete a variety of immunoregulatory cytokines.[3-5] LCs, which constitute about 2% of epidermal cells, express membrane ATPase, Fc and C^3 receptors, T6/Leu 6 (CD1a) antigen, and S-100 protein, and apart from acrosyringeal keratinocytes, are the only cells in normal epidermis which express Ia (immune response associated) or HLA-DR class II major histocompatibility complex (MHC) antigen. Antigen-presenting LCs play a critical role in the development of cutaneous immune reactions including allergic contact dermatitis, since they induce mitogenic, allogeneic and antigen-specific T cell activation and the generation of cytotoxic T cells.[6-11] The best characterized of the cytokines secreted by keratinocytes, termed epidermal cell-derived thymocyte activating factor (ETAF), has biological properties very similar to interleukin 1 (IL-1).[5,12] Studies in rodents[2,13-16] and humans[15,17] have shown that topical corticosteroids cause a dose-related reversible decrease in LC surface markers (including membrane ATPase, Fc and C_3 receptors, Ia antigen, T6 antigen and S-100 protein). Daily application of triamcinolone acetonide 0·1% for 5 days to the ears of mice resulted in decreased LC antigen-presenting capacity.[16] However, extrapolation of results of functional studies in so-called steroid-sensitive species, such as the mouse, to the immunoregulatory effect of *in vivo* administered corticosteroids in humans (a steroid-resistant species) should be undertaken cautiously.[18] The only reported study on the effects of steroids on the function of human LCs was carried out entirely *in vitro* using steroid concentrations well above pharmacological levels,[19] and the findings may not reflect adequately the effects of pharmacological doses of topical corticosteroids as used in clinical practice. We, therefore, investigated the effects of a range of commonly used topical corticosteroid agents on the antigen-presenting function of LCs in man.

METHODS

Subjects and treatments

Thirty one normal volunteers and 11 patients with psoriasis were studied; informed consent was obtained from all individuals. Subjects applied clobetasone butyrate 0·05% (Eumovate®; EV), betamethasone valerate 0·1% (Betnovate®; BV) or clobetasol propionate 0·05% (Dermovate®; DV) ointments to localized areas, 3 cm diameter, of clinically normal skin on the flexor aspect of one forearm, and inactive control vehicles to the contralateral forearm, twice daily for 7 days. Thus, each subject served as his or her own control. Tubes containing steroid or inactive vehicle were weighed before and at the end of the treatment period; the mean quantity of steroid or vehicle applied was 1·25 g per treatment site. Epidermal sheets free of dermal contamination were then harvested by the suction blister method as previously described, and were divided for use either in immunofluorescence studies or for processing to obtain suspensions of disaggregated epidermal cells.[20]

Serological reagents

The following monoclonal antibodies were used (see Table 1): fluoresceinated OKT6 (Ortho Diagnostic Systems Inc., High Wycombe, Bucks, U.K.) and unconjugated and phycoerythrin-conjugated anti-HLA-DR (Lab Impex Ltd., Teddington, Middx, U.K.). TU39 antibody

TABLE 1. Antibodies used for immunofluorescence staining of epidermal sheets

Antibody	Source	Dilution
Fluoresceinated OKT6	Ortho Diagnostic Systems Inc.	1:20
Phycoerythrin-conjugated anti-HLA-DR	Lab Impex Ltd.	1:20
Unconjugated anti-HLA-DR	Lab Impex Ltd.	1:50
TU39 (anti-class II MHC antigen)	Supplied by Dr M. Feldman	1:50
Fluoresceinated anti-mouse IgG (IgG fraction)	Dako Ltd.	1:20

directed against class II MHC antigen was kindly provided by Dr M. Feldmann, Charing Cross Sunley Research Centre, London. Fluorescein-conjugated IgG fraction of rabbit anti-mouse IgG was obtained from Dako Ltd., High Wycombe, Bucks, U.K. Serum from young rabbits was used as a source of complement.

Immunofluorescence staining

Epidermal sheets obtained from suction blister roofs were fixed and stained by an immunofluorescence method as previously described.[20] In the two-step procedure, the sheets were incubated with unconjugated anti-HLA-DR or TU39, diluted in Dulbecco's phosphate buffered saline (PBS; Gibco UK Ltd., Paisley, Scotland), for 16 h at 37°C, followed, after washing, by fluoresceinated rabbit anti-mouse IgG for 1 h. For double fluorescence staining with fluoresceinated OKT6 and phycoerythrin-conjugated anti-HLA-DR, a one-step method was used. The antibodies were combined in optimal dilutions in PBS containing 1% fetal bovine serum, and incubated with the epidermal sheet for 5 h at 37°C. Epidermal sheets were then mounted en face and examined using a Leitz EB22 microscope equipped with epifluorescence, and the mean number of dendritic LCs per mm^2 staining with fluorescein alone, phycoerythrin alone, or both fluorochromes was determined by counting five random fields of area 0·1 mm^2 with the use of an ocular grid. This number is referred to as the density of LC.

Preparation of cells

Peripheral blood mononuclear leukocytes (PBMNL) were obtained by centrifugation of heparinized venous blood over a Ficoll-Hypaque (Gibco UK Ltd.) density gradient. Purified T cells depleted of HLA-DR+ cells were obtained for use in the autologous epidermal–lymphocyte reaction by passage of PBMNL suspensions through a nylon wool column[21] followed by treatment with diluted anti-HLA-DR antibody for 30 min at 4°C, and then a 1:20 dilution of fresh rabbit serum as a source of complement.

Epidermal cell suspensions were obtained free of dermal cells following incubation of suction blister roofs with 0·5% tryspin (U.S. Biochemical Co., Cleveland, OH, U.S.A.) in PBS as previously described.[20,22,23] Epidermal cell viability, as determined by trypan blue exclusion, ranged between 90 and 95%.

Epidermal–lymphocyte reactions

Epidermal–lymphocyte reaction (ELR) assays were carried out as described previously.[22,23] Briefly, for the allogeneic ELR, stimulator epidermal cells from control and steroid-treated sites were cultured with 5×10^4 unfractionated allogeneic responder PBMNL in 96 well round bottom microculture plates (Nunclon, Gibco UK Ltd.) for 7 days in humidified air containing 5% CO_2. The culture medium consisted of RPMI 1640 (Gibco UK Ltd.) with added antibiotics, 2 mM L-glutamine and 15% human AB serum (Sigma Chemical Co., Poole, Dorset, U.K.). A range of from 2×10^5 to 2×10^4 epidermal cells was added to culture wells in order to obtain an overall profile of allostimulatory capacity, since the concentration of stimulator epidermal cells at which optimal or peak proliferation in the ELR occurs is variable. Wells with responder PBMNL alone were used to determine background counts. In some experiments, indomethacin 1 μg/ml (Sigma Chemical Co.), or recombinant interleukin 1 beta (kindly provided by Dr M. Feldmann) at a final concentration of 10 U/ml, were added to the wells. In other experiments, varying concentrations of epidermal cells from DV-treated sites were added to wells already containing control epidermal cells, in order to determine whether such addition resulted in inhibition of the control ELR.

For the autologous ELR, 5×10^4/well purified T cells, obtained from PBMNL suspensions depleted of HLA-DR+ cells, were used as the responder cells, and were cultured with varying numbers of autologous epidermal cells. For certain experiments, concanavalin A (Con A; Cambridge Bioscience, Cambridge, U.K.) was added to the wells at a final concentration of 80 μg/ml; in other experiments where subjects were known to have received vaccination against tetanus, tetanus toxoid (a gift from Dr M. Feldman) was added at a final concentration of 10 μg/ml. Wells containing responder PBMNL alone, to which were added Con A or tetanus toxoid, were used to determine background counts.

Cultures were pulsed with 1·0 μCi/well of tritiated thymidine (^{3}H-TdR; Amersham International plc, Amersham, UK; specific activity 25 Ci/mmol) for the last 18 h, and were harvested on a cell harvester (Flow Labs., Rickmansworth, Herts, U.K.); ^{3}H-TdR incorporation was then assessed by liquid scintillation counting. Results are expressed as the means of triplicate cultures.

Statistical analysis

The statistical significance of the results was analysed using Student's *t*-test or paired *t*-test as appropriate.

RESULTS

Immunofluorescence studies

Immunofluorescence examination of epidermal sheets stained with TU39 antibody, or anti-HLA-DR antibody, followed by rabbit anti-mouse IgG, or with phycoerythrin-conjugated anti-HLA-DR antibody or fluoresceinated OKT6 antibody, either alone or in combination, revealed a marked decrease in the number of class II MHC antigen- and T6 antigen-positive LCs following topical corticosteroid therapy, which was statistically significant for all three steroids ($P < 0{\cdot}05$) (Figs 1 and 2). Residual HLA-DR+/T6+ LCs appeared larger than control LCs, with longer dendrites (Fig. 1). The control density of LCs ranged from 530 to 937 cells/mm^2 (mean 672 cells/mm^2); following steroid therapy, the density of HLA-DR+/T6+ LCs was reduced in a potency-related manner (Fig. 2). Statistical analysis showed that there was no significant difference between the reductions in HLA-DR+/T6+ LCs following EV and BV

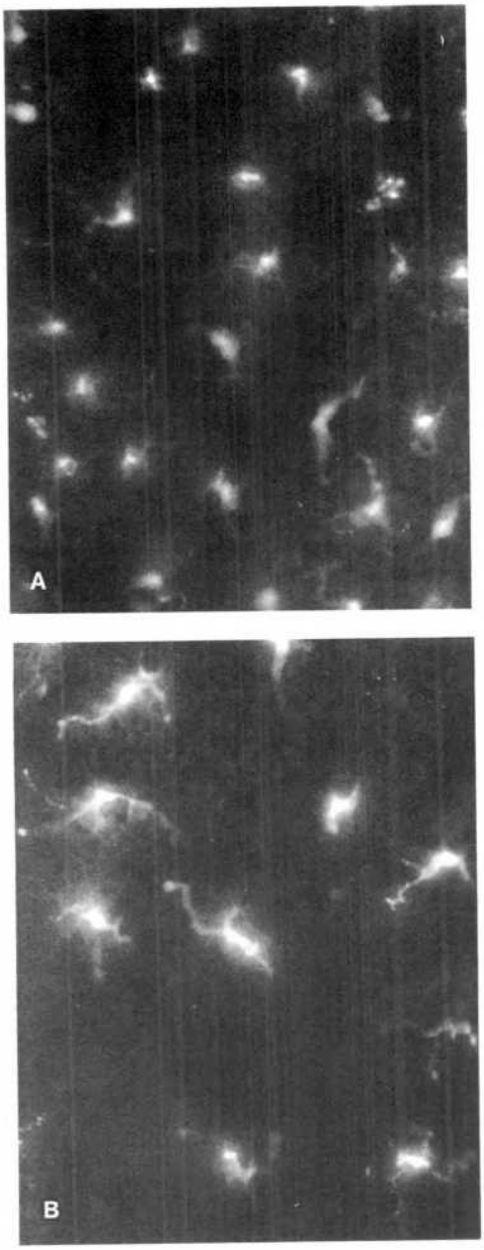

FIGURE 1. Immunofluorescence staining of epidermal sheets with phycoerythrin-conjugated anti-HLA-DR. (A) Control; (B) following therapy with clobetasol propionate 0·05% (Dermovate®) (original × 320).

therapy, but the reduction after DV therapy was significantly greater than that following the other two therapies ($P < 0{\cdot}001$).

In experiments using the double immunofluorescence staining technique, it was determined that, in both control and steroid-treated skin, all HLA-DR+ LCs were also T6+ and vice

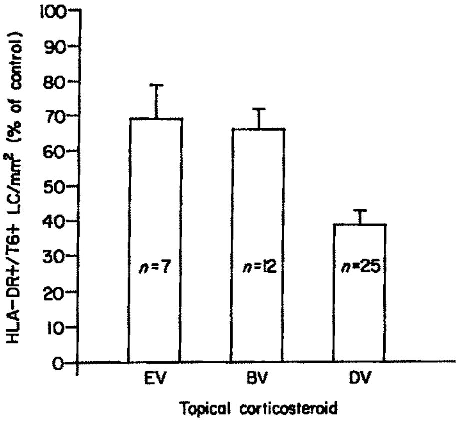

FIGURE 2. Density of HLA-DR+/T6+ Langerhans cells in epidermal sheets following topical steroid therapy, expressed as a percentage of control. Bars are means ± SEM. EV: clobetasone butyrate 0·05%, BV: betamethasone valerate 0·1%, DV: clobetasol propionate 0·05%.

versa; in other words, cells which were either HLA-DR+/T6−, or HLA-DR−/T6+ were not seen.

Steroid therapy decreases epidermal cell alloantigen-presenting capacity

Optimal allostimulation in the control ELR occurred at stimulator epidermal cell concentrations of 5×10^4 or 1×10^5 per well. In experiments for which 5×10^4 epidermal cells/well was the optimal concentration, higher concentrations of epidermal cells resulted in decreased T cell proliferation. The alloantigen presenting capacity in the ELR of epidermal cells from steroid-treated sites was significantly decreased compared with that of epidermal cells from control sites, for all three steroids ($P < 0·01$) (Figs 3 and 4). This was particularly evident at the higher epidermal cell concentrations, which induced optimal stimulation in the control ELR. The results of a representative experiment, carried out in one individual, showing the effects of clobetasol propionate 0·05% (DV) on the ELR curve are given in Figure 3. Analysis and comparison of the effects of EV, BV and DV on the allogeneic ELR in a large number of subjects shows that topical corticosteroid therapy results in a marked, potency-related reduction in the capacity of epidermal cells to present alloantigen; because the absolute values of ^{3}H-TdR incorporation, and the concentration of control epidermal cells resulting in optimal responder lymphocyte proliferation, varied considerably between experiments, results are expressed as a percentage of the optimal ^{3}H-TdR incorporation induced by control epidermal cells (Fig. 4). There was a trend suggesting that DV therapy depressed allostimulation to a greater extent than did EV or BV, although this was statistically significant only for EV ($P < 0·05$).

Steroid therapy decreases epidermal cell antigen-presenting capacity in the autologous ELR

In order to investigate further the effects of topical corticosteroid therapy on antigen presentation, we studied the capacity of epidermal cells from steroid-treated sites to present antigen in the autologous ELR. Clobetasol propionate 0·05% markedly reduced the capacity of epidermal cells to induce Langerhans cell-dependent augmentation of Con A-stimulated lymphocyte proliferation, and to present tetanus toxoid to sensitized autologous lymphocytes (Fig. 5).

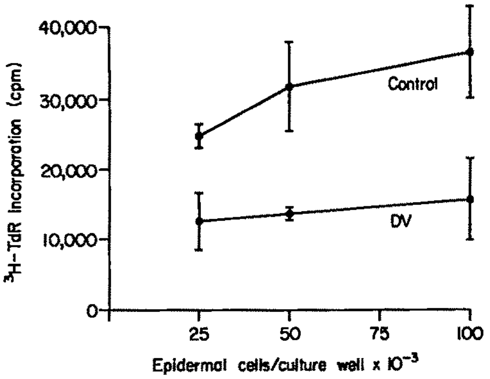

FIGURE 3. Effect of topical therapy with clobetasol propionate 0·05% (DV) on the allostimulatory capacity of epidermal cells in the allogeneic ELR. Results of a representative experiment showing ELR curves. Points are means ± SEM.

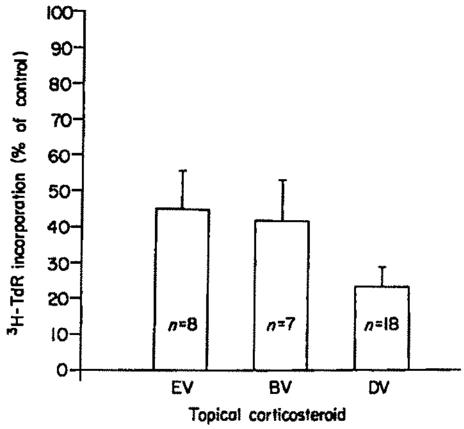

FIGURE 4. Effect of topical corticosteroid therapy on the allostimulatory capacity of epidermal cells in the allogeneic ELR. The concentration of control epidermal cells (5×10^4 or 1×10^5 per well) resulting in optimal responder lymphocyte proliferation was determined for each experiment from the ELR curve. Results were derived by expressing the ^{3}H-TdR incorporation induced by epidermal cells from treated sites at this concentration as a mean percentage (± SEM) of the optimal ^{3}H-TdR incorporation induced by control epidermal cells. EV: clobetasone butyrate 0·05%, BV: betamethasone valerate 0·1%, DV: clobetasol propionate 0·05%.

Decreased allostimulatory capacity of epidermal cells after steroid therapy is not restored by exogenous interleukin 1

Addition of exogenous IL-1 to allogeneic ELR cultures did not restore the decreased alloantigen presenting capacity of epidermal cells from sites treated with clobetasol propionate 0·05% (Figs 6 and 7), indicating that the decrease was not primarily the result of diminished endogenous ETAF production.

Decreased allostimulatory capacity of epidermal cells after steroid therapy is not restored by indomethacin

Addition of indomethacin to the culture medium resulted in increased T cell proliferation in

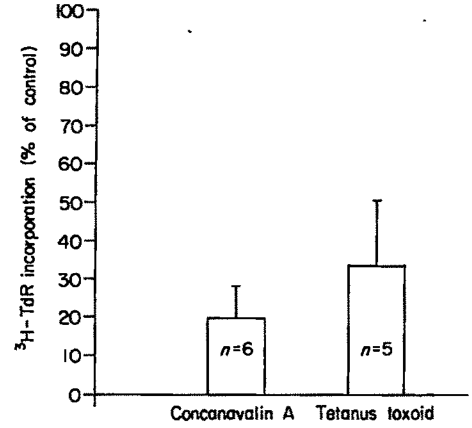

FIGURE 5. Effect of topical therapy with clobetasol propionate 0·05% on the capacity of epidermal cells to augment Con A-stimulated lymphocyte proliferation, and to present tetanus toxoid, in the autologous ELR. Results were derived by expressing the ^{3}H-TdR incorporation induced by epidermal cells from treated sites as a mean percentage (± SEM) of the optimal ^{3}H-TdR incorporation induced by control epidermal cells.

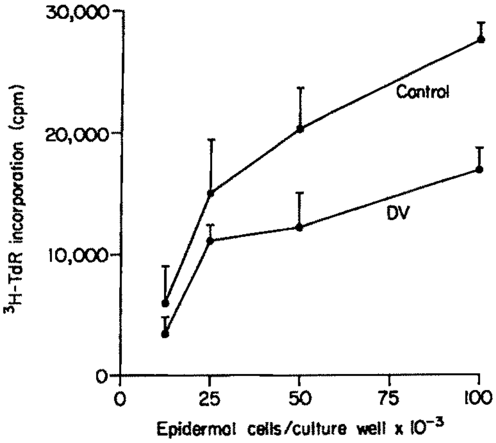

FIGURE 6. Effect of addition of interleukin 1 on the allostimulatory capacity of epidermal cells in the allogeneic ELR following topical therapy with clobetasol propionate 0·05%. Results of a representative experiment showing ELR curves. Points are means ± SEM.

response to epidermal cells from both control and steroid-treated sites (data not shown). Indomethacin did not, however, restore the relative decrease in allostimulatory capacity of epidermal cells from sites treated with clobetasol propionate 0·05%, compared with control epidermal cells, in the allogeneic ELR (Fig. 7), indicating that this decrease was not the result of the production of inhibitory prostaglandins by epidermal cells during the culture period.

Effect of addition of epidermal cells from steroid-treated sites on the control ELR

In order to determine whether the observed decrease in allostimulatory capacity following steroid therapy might be the result of inhibitory factors other than prostaglandins, we investigated the effect on the control ELR of addition of epidermal cells from sites treated with

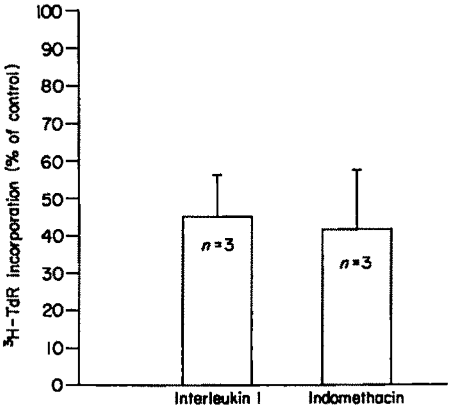

FIGURE 7. Effect of addition of interleukin 1 or indomethacin to the culture medium on the allostimulatory capacity of epidermal cells following topical therapy with clobetasol propionate 0·05%. Results were derived by expressing the ^{3}H-TdR incorporation induced by epidermal cells from treated sites as a mean percentage (± SEM) of the optimal ^{3}H-TdR incorporation induced by control epidermal cells.

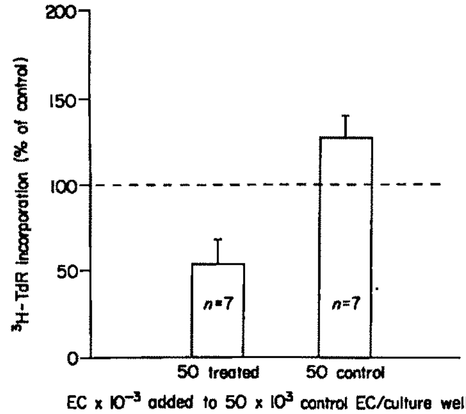

FIGURE 8. Effect of addition of epidermal cells from steroid-treated sites on the control ELR. The histogram shows the effect of adding a further 5×10^4 epidermal cells/well, from either control sites or from sites treated with clobetasol propionate 0·05%, on the allostimulatory capacity of 5×10^4 control epidermal cells. Results are expressed as the mean percentage (± SEM) of the ^{3}H-TdR incorporation induced by 5×10^4 control epidermal cells alone.

clobetasol propionate 0·05%. Control or steroid-treated epidermal cells (5×10^4) were added to a fixed concentration of 5×10^4 control epidermal cells, giving a total of 1×10^5 epidermal cells/well. Whereas addition of control epidermal cells resulted in increased allostimulation, lymphocyte proliferation was decreased when epidermal cells from steroid-treated sites were added (Fig. 8). These findings indicate that the decreased allostimulatory capacity of epidermal cells from steroid-treated sites compared with control was the result of active suppression of the ELR.

DISCUSSION

Glucocorticosteroids exert a variety of immunosuppressive effects on both lymphocytes and monocyte-macrophages.[18,24] *In vitro* testing has revealed that steroids inhibit mitogen-stimulated,[25] alloantigen-stimulated, and antigen-specific, T lymphocyte proliferation,[26,27] perhaps by inhibiting interleukin 2 production,[28] and suppress the generation, proliferation and effector function of cytotoxic T lymphocytes.[29,30] Steroids block the action of macrophage inhibitory factor on the macrophage,[31] and inhibit Ia antigen expression, IL-1 production, and antigen presentation by murine peritoneal macrophages.[32] Methylprednisolone and hydrocortisone have been reported to cause reversible inhibition of antigen presentation by human peripheral blood monocytes and endothelial cells *in vitro*, but only in concentrations exceeding pharmacological levels.[26] Hydrocortisone-mediated inhibition of human monocyte antigen presentation occurs only if steroid is present during monocyte antigen pulsing, indicating that it interferes with some aspect of antigen presentation such as antigen uptake, processing, or association with Ia antigens; inhibition is not overcome by addition of IL-1, and occurs despite the fact that hydrocortisone increases class I and class II MHC (HLA-DR) expression by human monocytes.[27,33] Hydrocortisone reportedly did not affect IL-1 production by human monocytes, but appeared to act as an antagonist of the effect of IL-1.[33] In addition to interfering with the function of lymphocytes and macrophages as described above, systemic glucocorticosteroid therapy may exert an immunosuppressive effect by altering the kinetics and distribution of these cell types.[18,24]

We attempted to analyse the effects of pharmacological doses of corticosteroids widely used in dermatological practice on resident antigen-presenting, macrophage-like LCs in the epidermis, in isolation from any potential steroid effects on lymphocytes. We found that topical corticosteroid therapy caused a statistically significant, potency-related decrease in the number of LCs expressing HLA-DR and T6/CD1a antigens. In contrast to previous reports in which topical amcinonide or betamethasone 17-valerate 0·05% selectively decreased LC Ia antigen,[15,17] in our study T6 antigen expression was decreased to the same extent as HLA-DR expression. We also demonstrated that topical steroid therapy in man induces a statistically significant fall in the allostimulatory capacity of epidermal cells in the allogeneic ELR, as well as a considerable reduction in Con A-stimulated and tetanus toxoid-specific lymphocyte proliferation in the autologous ELR. The extent of reduction in the number and allostimulatory capacity of HLA-DR+/T6+ LCs following therapy with the three steroid ointments paralleled their relative potencies as assayed using the standard occluded vasoconstrictor technique.[34] Enumeration of HLA-DR+/T6+ LCs and assay of their allostimulatory capacity may prove to be a useful additional technique in the classification of topical corticosteroids according to their potency.

Corticosteroids have been reported to inhibit IL-1 secretion by murine macrophages,[32] and to antagonize the action of IL-1 in man.[33] The decreased epidermal cell allostimulatory capacity following steroid therapy was not, however, primarily the result of diminished endogenous ETAF production by keratinocytes, since addition of exogenous IL-1 to ELR cultures did not restore allostimulation. Prostaglandin E_2 secreted by keratinocytes in culture is well known as a potential cause of inhibition of lymphocyte proliferation in the ELR assay.[35,36] However, glucocorticoids reduce the biosynthesis of prostaglandins via the induction of a group of phospholipase A_2 inhibitory proteins, collectively termed lipocortin.[37] We excluded the possibility that the steroid-induced diminution in allostimulatory capacity in the ELR was the result of a rebound synthesis of prostaglandin E_2 during the 7-day culture period following cessation of steroid therapy, by demonstrating that allostimulation was not enhanced by addition of indomethacin to the culture medium.

Since LCs are the critical accessory/stimulator cells in the allogeneic and autologous

ELR,[6–11] and since the decrease in epidermal cell allostimulatory capacity paralleled the fall in HLA-DR+/T6+ LCs, our findings might be interpreted as evidence that topical corticosteroid therapy depresses antigen presentation by epidermal cells by a direct effect on the antigen-presenting function of LCs, as previously suggested on the basis of murine experiments.[16] This would be in keeping with reports that steroids inhibit antigen processing by human macrophages,[27,33] and in suprapharmacological doses reversibly decrease the capacity of LCs to present specific antigen to sensitized T cells.[19] It might be postulated that the apparent decrease in LC antigen-presenting capacity is simply the result of a decrease in LC HLA-DR expression. However, previous studies of corticosteroid action have shown that there is no simple correlation between changes in class II MHC antigen expression and changes in the effectiveness of antigen presentation;[27,33] in particular, signals which down-regulate Ia expression may have little effect on antigen presentation, perhaps because T lymphocytes require only low levels of class II MHC antigen expression by antigen presenting cells.[38]

The fact that epidermal cells from steroid-treated sites reduced the allostimulatory capacity of control epidermal cell suspensions indicates that this decreased allostimulatory capacity involves active suppression of the ELR, and is not purely due to decreased LC antigen presenting function. Even though epidermal cell suspensions were washed extensively before use in the ELR, we cannot entirely exclude the possibility that steroid was carried over into the cultures, and could have directly inhibited T cell proliferation. Although reports on steroid effects on suppressor cell generation and function have been conflicting,[30,39] low doses of steroid have been reported in one study to potentiate the effect of alloantigen-induced suppressor cells,[30] so that if inhibition were the result of steroid carryover, it might have involved suppressor T cell activity rather than a direct steroid effect. However, pre-incubation of epidermal cells with suprapharmacological doses of methylprednisolone for 90 min *in vitro*, a situation in which steroid carryover might have been expected to occur, did not reduce the subsequent allostimulatory capacity of LC in the allogeneic ELR.[19] An alternative explanation for the suppression of the ELR is that steroid therapy might result in the production of an inhibitory substance by keratinocytes, such as epidermal cell-derived lymphocyte differentiating factor (ELDIF).[40] Whatever the mechanism involved in the suppression of the ELR, it is clear that topical steroid therapy in man decreases the number of HLA-DR+/T6+ LCs, and markedly inhibits T cell activation normally subserved by LCs. In the absence of reports on statistical analysis of the number of LCs identified ultrastructurally before and after steroid therapy, the question as to whether steroid therapy destroys LCs, or merely modulates expression of LC surface markers, has been the subject of considerable debate.[13–17,41] Since the local proliferative capacity of LCs is generally held to be low, the return of Ia+/HLA-DR+ LCs following cessation of steroid therapy[14–17,41] could be the result of replenishment from a circulating pool derived from the bone marrow, or of re-expression of Ia/HLA-DR antigen. The fact that steroid-induced depletion of HLA-DR+ LCs is reversed with time even when human skin is transplanted to athymic (nude) mice, a system devoid of circulating precursors for human LCs, strongly supports the view that steroids induce modulation of LC markers rather than LC death.[41]

ACKNOWLEDGMENTS

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Increased serum levels of squamous cell carcinoma-related antigen in pemphigus

H.KUDO, M.YAMAMOTO, Y.MIYACHI, K.DANNO AND S.IMAMURA

Department of Dermatology, Kyoto University Faculty of Medicine, Kyoto, Japan

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SUMMARY

Serum levels of squamous cell carcinoma-related antigen (SCC-RAG) were measured in five cases of pemphigus, five cases of bullous pemphigoid and 18 cases of benign and malignant dermatoses other than SCC. The SCC-RAG titres were significantly raised in four of five patients with pemphigus, while they remained within the normal range in the other dermatoses except in one case. In three pemphigus cases in whom serial measurements were made, SCC-RAG levels seemed to be related to disease activity. The SCC-RAG levels in blister fluids were much higher than those in serum, suggesting that the skin is a major source of serum SCC-RAG.

These results show that SCC-RAG is increased not only in SCC, but also in some cases of pemphigus, and suggest that pemphigus antibodies may cause the production or release of SCC-RAG.

The tumour antigen TA-4, derived from squamous cell carcinoma (SCC) of the uterine cervix, provides a useful marker for cervical SCC using a radioimmunoassay system.[1,2] One of the subfractions of TA-4 termed SCC-related antigen (SCC-RAG) was later extracted from metastatic lesions of cervical SCC. The SCC-RAG radioimmunoassay kit is now commercially available from Dainabot Laboratories and according to clinical studies using this kit, serum levels of SCC-RAG are significantly raised not only in cervical SCC but also in lung, oesophageal and cutaneous SCC compared with negligible titres in normal subjects.[3–5]

However, recent studies have shown that serum levels of SCC-RAG are also high in some patients with non-cancerous lung diseases. In these cases, the SCC-RAG may be derived from inflammatory lung tissues. Immunohistological studies have found SCC-RAG to be located not only in SCC, but also in normal stratified squamous epithelia of the cervix[6], lung[7], and skin (H. Kudo *et al.*, unpublished data). In pemphigus, anti-intercellular substance (ICS) antibodies bind to keratinocytes with resultant acantholysis. The present study was carried out to

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Correspondence: Yoshiki Miyachi, Department of Dermatology, Faculty of Medicine, Kyoto University, 54 Shogoin Kawahara-cho, Sakyo-ku, Kyoto 606, Japan.

determine whether patients with pemphigus had elevated serum levels of SCC-RAG, whether serum levels of SCC-RAG correlated with disease activity and whether or not the skin is a major source of serum SCC-RAG.

METHODS

Titres of serum SCC-RAG were assayed in five cases of pemphigus, five cases of bullous pemphigoid, 11 cases of other benign dermatoses including psoriasis vulgaris (2), herpes zoster (3), dermatomyositis (4), exfoliative dermatitis (1), and linear IgA bullous dermatosis (1), and 7 cases of malignant dermatoses including malignant melanoma (3), Paget's disease (2), cutaneous T cell lymphoma (1), and multiple Bowen's disease (1). Levels of SSC-RAG in blister fluids were also measured in duplicate in each case of pemphigus and pemphigoid. The diagnoses of pemphigus and bullous pemphigoid were made on the basis of clinical, histological and immunofluorescence findings. All five cases of pemphigus showed positive intercellular staining on both direct and indirect immunofluorescence. In Cases 1, 4 and 5, histology showed acanthosis of the upper epidermis and the diagnosis was pemphigus foleaceus. In Case 2 a suprabasal cleft was found and the diagnosis was pemphigus vulgaris. Case 3 showed only eosinophilic spongiosis in the absence of a suprabasal cleft. Because of a clinical resemblance to dermatitis herpetiformis, this case was diagnosed as so called herpetiform pemphigus.

Determination of SCC-RAG levels was performed using a radioimmunoassay kit (Dainabot Laboratories, Tokyo, Japan), according to the manufacturer's instructions. Briefly, serum or blister fluid samples were incubated with ^{125}I-SCC-RAG and anti-SCC-RAG rabbit antiserum. Then the second antibody solution (goat anti-rabbit gamma globulin) was added and the mixture was incubated again. Antigen-antibody complexes were separated from free ^{125}I-SCC-RAG by centrifugation at 1800 *g* for 20 min and radioactivity in the pellet was counted in a gamma counter. The amount of SCC-RAG in the samples was estimated from a standard curve.

Titres of pemphigus antibody were determined semi-quantitatively using an indirect immunofluorescence method.

RESULTS

The mean level of serum SCC-RAG ($\pm$ SE) was significantly higher in patients with pemphigus ($5{\cdot}68 \pm 1{\cdot}91$ ng/ml) than in patients with bullous pemphigoid ($1{\cdot}10 \pm 0{\cdot}14$ ng/ml) ($P < 0{\cdot}05$) and the control subjects with other dermatoses ($1{\cdot}48 \pm 0{\cdot}27$ ng/ml) ($P < 0{\cdot}001$; Students *t*-test) (Fig. 1). Only one of the control subjects (with herpes zoster) had a serum level of SCC-RAG slightly in excess of 2·0 ng/ml, which was taken as the upper limit of the normal range.

We examined the relationship between the titres of pemphigus antibody and the levels of serum SCC-RAG in the patients with pemphigus (Fig. 2). SCC-RAG titres tended to be related to pemphigus antibody titres except in one case, although statistical analysis was not performed because of the small number of cases.

In Cases 1, 2 and 3, serum SCC-RAG levels were determined on several occasions. SCC-RAG levels declined to within the normal range after remission in Cases 1 and 2, but in Case 3, where remission was not obtained, SCC-RAG levels remained high. In none of these cases was evidence of internal carcinoma found on systemic examination including whole body CT scanning and ^{67}Ga scintigraphy.

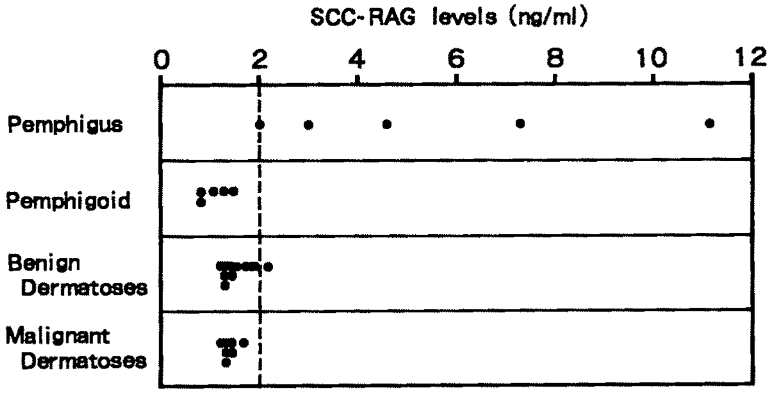

FIGURE 1. Serum levels of SCC-RAG in pemphigus, bullous pemphigoid and other dermatoses.

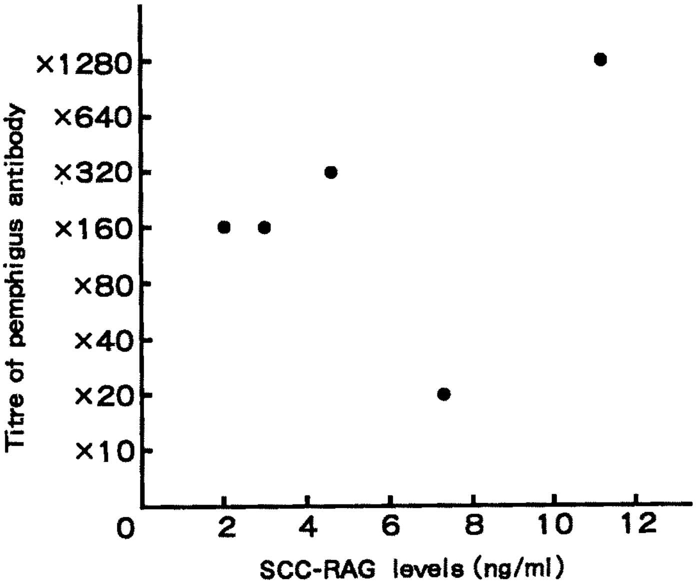

FIGURE 2. Relationship between SCC-RAG levels and the titre of pemphigus antibody, in five patients with pemphigus measured by an indirect immunofluorescence method.

The SCC-RAG levels in blister fluid were much higher than those measured in serum. The level was above 1500 ng/ml in one case of pemphigus (Case 1) and 326 ng/ml in one case of bullous pemphigoid, compared with serum levels of 11·2 and 1·3 ng/ml, respectively.

DISCUSSION

Four of the five patients with pemphigus showed significantly raised levels of serum SCC-RAG; these were fourfold higher than those in the control subjects with other dermatoses including patients with bullous pemphigoid who had similarly low SCC-RAG levels to those previously reported in healthy subjects.[3] These results contrast with the previous report that in patients with non-cancerous pulmonary diseases, only two of 493 cases had serum SCC-RAG levels in excess of 5 ng/ml.[7] Furthermore, the finding of very high levels of SCC-RAG in blister fluids suggests that serum SCC-RAG originates mainly from the skin.

Because normal levels of SCC-RAG were found in patients with bullous pemphigoid who had a similar disease extent to those with pemphigus, chronic cutaneous erosions and ulcerations may not be the cause of the elevation of serum SCC-RAG. The difference in serum levels of SCC-RAG between pemphigus and bullous pemphigoid appears to result from the difference in the site or the mechanism of bulla formation. It is possible that SCC-RAG present in epidermal cells diffuses more easily into intraepidermal bullae than into subepidermal bullae. Alternatively, anti-ICS antibodies may cause the production or release of SCC-RAG.

While it has been shown by Yagi *et al.*[5], that SCC-RAG is a useful marker for detection and monitoring of internal SCC or metastasis of skin SCC, results must be interpreted with caution in cases of pemphigus and pemphigus-like diseases. Pemphigus may also provide a useful clinical model for investigating the mode of production and secretion of SCC-RAG by epidermal cells.

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Effect of BN 52021, a platelet activating factor antagonist, on experimental murine contact dermatitis

M.CSATÒ AND B.M.CZARNETZKI

Department of Dermatology, University of Münster, von Esmarch-Strasse 56, D-4400 Münster, F.R.G.

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SUMMARY

A specific platelet activating factor (PAF) receptor antagonist, BN 52021, was tested for its efficacy in modulating DNFB-induced allergic and croton oil-induced irritant contact dermatitis in mouse ears. Oral treatment of the animals in the elicitation phase of the dermatitis caused a significant suppression of DNFB-induced ear swelling. The compound was less active in croton oil-induced irritant dermatitis and ineffective when given during the sensitization phase of allergic dermatitis.

The results provide indirect evidence for the involvement of PAF in the effector phase of murine contact dermatitis of the allergic type and also the irritant type to a lesser extent.

Inflammation of the skin is characterized by increased vascular permeability, plasma protein exudation and immigration of inflammatory cells. All these characteristics of inflammation are readily induced by the unique ether phospholipid, platelet activating factor (PAF).[1] For this reason, PAF has recently received increasing attention as a potential mediator of cutaneous pathology.

Intradermal injection of PAF-acether in experimental animals and in humans results in an acute weal and flare response followed by erythema.[2,3] PAF and PAF-like ether lipids have been identified in inflammatory skin diseases, such as psoriasis[4] and cold urticaria.[5] Although the source of PAF found in these disorders is unclear, these observations further support the potential significance of PAF in cutaneous pathology.

One possible approach to clarification of the role of PAF in different pathological conditions is provided by the use of specific antagonists. Highly selective receptor antagonists have recently become available. BN 52021 (ginkgolide B), identified originally in *Ginkgo biloba* tree extracts, has been reported to be a potent PAF antagonist.[6] The compound inhibits all the biological

Correspondence: Professor Dr B.M. Czarnetzki, Clinical Research Dermatology, F. Hoffmann-La Roche & Co. Ltd. CH-4002 Basel, Switzerland.

activities induced by PAF *in vitro* and *in vivo* in laboratory animals (see Braquet *et al.*[7] for review), including inhibition of platelet aggregation, bronchoconstriction, hypotension and the chemotaxis and activation of polymorphonuclear and eosinophil leukocytes. A ginkgolide mixture, BN 52063, diminishes the weal and flare response of the skin to PAF and selectively inhibits *in vitro* PAF-induced platelet aggregation when administered orally to humans.[8]

Arachidonate-induced experimental murine contact dermatitis has been used to study the potential role of lipid mediators in inflammatory dermatoses.[9] Unfortunately, this model does not apply to any known human disease. We, therefore, chose instead the model of allergen- and croton oil-induced irritant contact dermatitis, with oral administration of the potent PAF antagonist BN52021, to study the possible involvement of PAF in these conditions.

METHODS

Female Balb/c mice were sensitized on two succeeding days with 15 μl of 0·5% dinitrofluorobenzene (DNFB) (Sigma, Munich, F.R.G.) in acetone/olive oil (4:1) on the razor shaved abdomen.[10] Three days later, allergic contact dermatitis was elicited by painting both ears with 20 μl of 0·3% DNFB. Irritant dermatitis was induced by a single application of 0·5% croton oil (Sigma, Munich, F.R.G) in acetone/olive oil (4:1) on both ears.

BN 52021 (kindly provided by Professor P. Braquet, Institut Henri Beaufour, Le Plessis Robinson, France), as a 2% suspension in gum arabic and water (1:10), was administered to the animals via a gastric feeding tube at a dose of 50 mg/kg body weight/day. This dose was used at the suggestion of Professor Braquet on the basis of good activity observed by him in other pathological animal models.

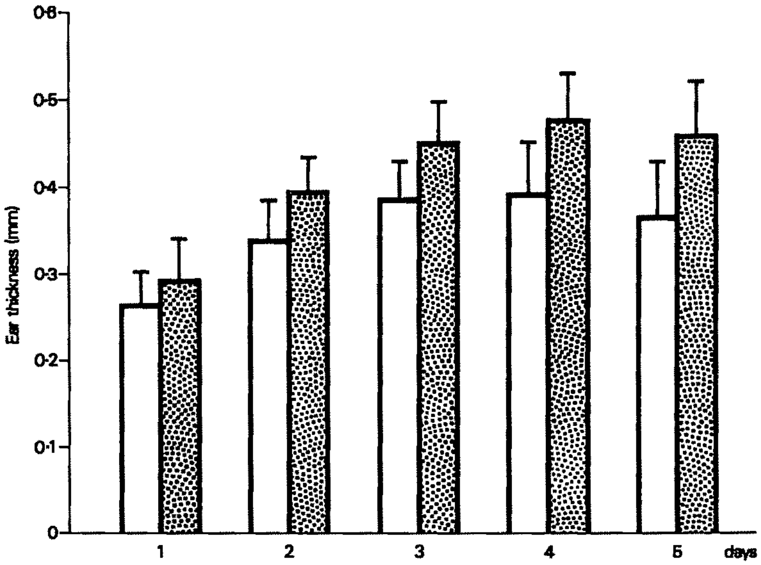

FIGURE 1. Ear thickness in DNFB sensitized animals measured at 4 h (day 1) and at 24 h intervals after elicitation. □ = animals treated with BN 52021. ▨ = control animals. Bars are means ± SD for groups of 58 animals. Ear thickness was significantly less in BN 52021 treated animals than in controls at all times of reading ($P < 0·05$).

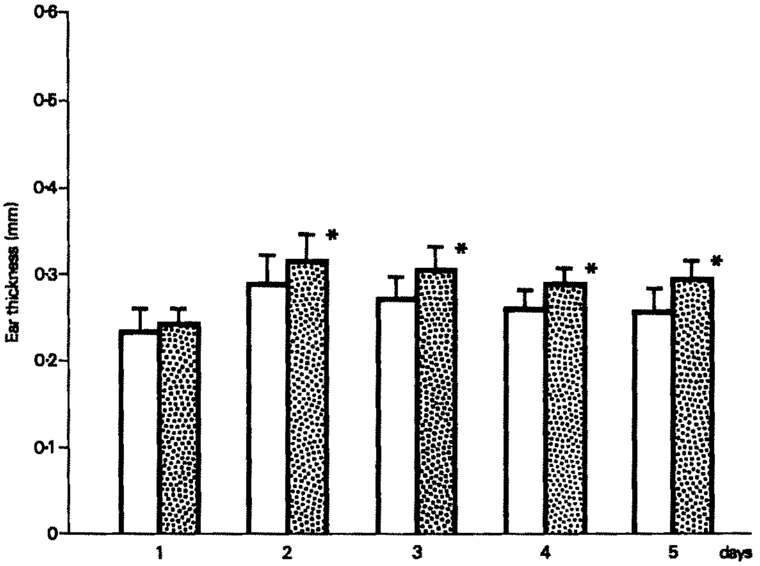

FIGURE 2. Ear thickness in animals with croton oil dermatitis. □ = BN 52021 treated animals, ▨ = controls. Bars are means ± SD for groups of 20 animals. * BN 52021 treated animals significantly less than controls ($P < 0{\cdot}05$).

Oral feedings of BN 52021 were administered 2 h before the elicitation of the dermatitis and repeated thereafter at 24 h intervals on 5 subsequent days. One series of animals was treated 1 day before and on the 2 days of allergic sensitization, but not during the elicitation phase. Sensitized and elicited animals without any drug treatment served as controls.

Ear thickness was measured with a spring loaded micrometer 1–3 h after the elicitation of the dermatitis and at 24 h intervals for up to 5 days.

Each experiment was repeated four times for treatment during elicitation and twice for treatment during sensitization, using groups of 20–58 animals.

Statistical analysis was carried out using Student's *t*-test. Results for BN 52021 treated animals were compared with results from their respective controls obtained on the same day.

RESULTS

Treatment with BN 52021 2 h prior to and at 24 h intervals after the elicitation of DNFB induced allergic contact dermatitis resulted in a significant reduction in the ear swelling of the animals. A difference in ear thickness between the treated and untreated groups was already present at 4 h after elicitation of the dermatitis and increased during the subsequent 4 days (Fig. 1).

Administration of BN 52021 to the animals with croton-oil induced irritant contact dermatitis resulted in a reduction of ear swelling at days 1-5. The reduction was statistically significant on days 2–5 (Fig. 2).

Treatment of the animals during the sensitization phase of allergic dermatitis had no significant effect on the subsequent elicitation phase (Fig. 3).

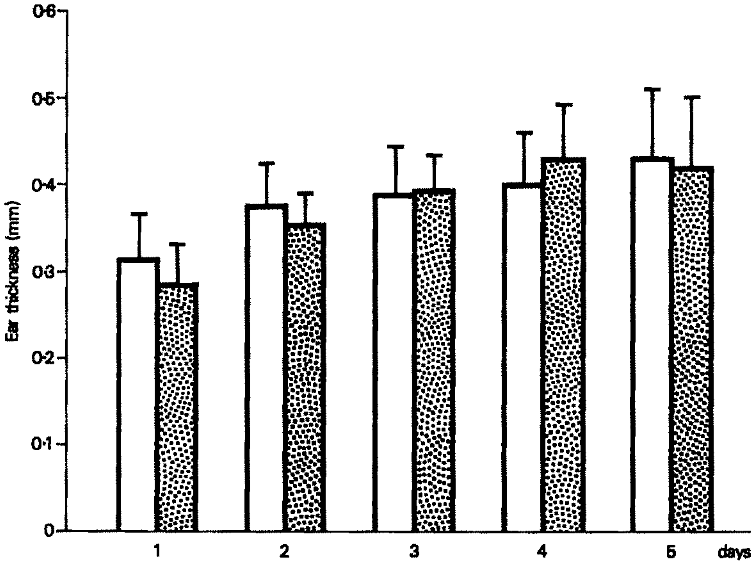

FIGURE 3. Ear thickness in animals who had been treated with oral BN 52021 during the sensitization phase of allergic contact dermatitis □, and in untreated controls ▨. No BN 52021 was given during elicitation. Bars are means ± SD for groups of 40 animals. The two groups did not differ significantly.

DISCUSSION

The present data demonstrate that the PAF antagonist BN 52021 is able to suppress murine experimental contact dermatitis during its elicitation phase. The effect is more prominent in DNFB-induced allergic contact dermatitis than in croton oil-induced irritant contact dermatitis.

These results provide indirect evidence for the involvement of PAF in murine contact dermatitis. The ear swelling that is measured in the animals is thought to be due to rapidly developing tissue oedema followed by immigration of inflammatory cells into the skin. BN 52021 reduced the ear swelling during the 5 days on which measurements were made. This is consistent with the fact that PAF affects not only the vascular endothelium and the exudation of vascular fluid, but also the chemotaxis of leukocytes[1,11]

The fact that the PAF antagonist BN 52021 was less effective in irritant than in allergic dermatitis is intriguing, particularly in view of the fact that croton oil caused less severe ear swelling than DNFB in sensitized animals. The peptidoleukotriene antagonist Ro 23-3544 which we tested earlier in the same model, was similarly less active in irritant dermatitis.[12] It is conceivable that different pathomechanisms in the two diseases account for the differing responsiveness to the antagonists, with cytotoxic effects predominating in irritant dermatitis because of the non-specific membrane-active, cytolytic effects of irritants. The present observations are in keeping also with previous observations[13] that a lipoxygenase is inhibited in allergic dermatitis and enhanced in irritant dermatitis in the guinea pig.

Treatment of the animals during the sensitization phase had no effect on the subsequent DNFB-induced dermatitis. The mechanisms accounting for and involved in immunological sensitization differ fundamentally from those operative in the elicitation phase of the dermatitis. Numerous effects of PAF on the antigen presenting immune cells *in vitro* have been reported (see Braquet et al.[6] for review), including the regulation of lymphocyte proliferation and the production of interleukins 1 and 2. These *in vitro* effects, however, may not be significant in the *in vivo* situation, as shown by the data in Figure 3.

Thus the present data provide evidence for the involvement of PAF in the elicitation of murine contact dermatitis. In a previous study, we have shown that a peptidoleukotriene antagonist was somewhat less effective in the same model.[12] Taken together, these findings may help in the understanding of the relative importance of mediators in contact eczema and might perhaps pave the way to better therapeutic modalities in this condition.

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Prognostic value of Langerhans cells in the epidermis of HIV patients

B.DRÉNO, B.MILPIED, J.D.BIGNON, J.F.STALDER AND P.LITOUX

Clinique Dermatologique, Hôtel-Dieu, C.H.U., Nantes, France

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SUMMARY

We measured the number of Langerhans cells in clinically normal epidermis and the number of T4 lymphocytes in the plasma in 24 patients infected with HIV, and related these to the stage of the disease. A correlation is demonstrated between the numbers of Langerhans cells and T4 lymphocytes. Numbers of Langerhans cells were lower in patients with disease stages III and IV than in those with disease stage II. Thus Langerhans cell numbers could be of prognostic value in this disease.

In HIV infection, it is believed that immunodeficiency results from the affinity of HIV for T helper cells[1] and subsequent destruction of these cells. However, some reports suggest that antigen-presenting cells may play an important role in the immunological abnormalities of this disease. HIV has been isolated from follicular dendritic cells[2], mononuclear phagocytes[3,4] and Langerhans cells,[5] and the number of Langerhans cells in the skin of patients infected with HIV has been found to be reduced.[6]

At the present time, the absolute number of T helper cells in the plasma is considered to be one of the best prognostic factors in assessing the course of HIV infection. In the present study, the number of Langerhans cells in normal skin of 24 patients was determined at different stages of HIV infection.

METHODS

Patients

Twenty-four patients with serologically proven HIV infection (22 men, two women; age range 18–55 years), who had received no immunosuppressive agents, corticosteroids, radiotherapy or photochemotherapy (PUVA) during the preceding year, were included in the study. The patients were classified according to the Centre for Disease Control (CDC) classification (1986) (Table 1). Six patients were classed as stage II (asymptomatic infection), eight stage III

Correspondence: B.Dréno, Clinique Dermatologique, Hôtel-Dieu, C.H.U., 44035 Nantes Cedex 01, France.

TABLE 1. Centre for Disease Control (CDC) classification of disease stages in HIV infection

Stage	Symptoms
I	Acute infection
II	Asymptomatic infection
III	Persistent generalized lymphadenopathy
IV A	Systemic features
IV B	Neurological disease
IV C	Secondary infections
IV D	Secondary cancers: Kaposi's sarcoma and lymphoma
IV E	Other conditions

(persistent generalized lymphadenopathy), six stage IV A (systemic features) and four stage IV D (secondary cancers), with Kaposi's sarcoma. Fourteen of the patients were homosexuals, five were drug addicts and four were heterosexuals. Controls were 10 healthy heterosexuals (five men, five women; age range 20–50 years) not in an AIDS risk group and with negative HIV serology.

T helper cells

Blood samples were taken from HIV patients and controls and lymphocytes isolated on a Ficoll gradient. The absolute number of T helper (T4) cells per mm^3 of plasma was determined by an indirect immunofluorescence technique, using the monoclonal antibody IOT4 (Immunotech) diluted 1:40. Counts were performed on 100 cells using a Leitz microscope at ×40 magnification.

Skin biopsies

Four mm punch biopsy specimens were obtained from clinically normal skin on the sun-protected inner side of the upper arm of the HIV patients and controls. The specimens were snap-frozen in liquid nitrogen and stored at −70°C.

Sections (3 μm) were cut, immersed for 10 min in acetone and washed for 10 min in phosphate buffered saline (PBS). The sections were incubated with monoclonal antibody IOT6 (diluted 1:10), which reacts specifically with epidermal thymocytes and Langerhans cells, for 20 min at 37°C. The sections were washed in PBS, and incubated with (Fab)$_2$ fluorescein-conjugated goat anti-mouse IgG (diluted 1:20) for 30 min at 37°C followed by washing in PBS. The sections were mounted in buffered glycerin and examined using a Zeiss microscope at ×300 magnification. The number of IOT6 positive cells in the epidermis was counted per mm length of epidermis. The mean number of Langerhans cells was determined in five fields (each 0·025 mm^2) in 3 sections of each biopsy. The sections were not consecutive to avoid counting the same cell in more than one section.

RESULTS

Numbers of T4 lymphocytes and Langerhans cells in the 24 patients in relation to the stage of HIV infection are listed in Table 2. The number of Langerhans cells in clinically normal epidermis was decreased in all the patients compared with the controls and no T6 cells were found in the dermis. However, T6 positive staining of keratinocytes was found in 20 patients,

TABLE 2. Numbers of epidermal Langerhans cells (LC) and plasma T4 cells in 24 HIV patients in relation to disease stage, and in normal controls

Disease stage	Case no.	Plasma T4 cells/mm³	LC/mm epidermal length	T6 positive keratinocytes*
II	5	440	9·0	–
	7	410	6·0	+
	17	982	9·2	++
	20	456	6·0	+
	23	477	5·0	–
	24	584	6·0	–
III	1	362	3·6	++
	2	470	6·0	–
	4	235	4·2	+
	8	236	3·8	+
	15	920	10·0	++
	16	162	7·0	+
	19	1200	9·0	+
	21	353	4·0	+
IV A	3	42	2·0	+++
	11	525	5·4	+
	12	450	3·0	+
	13	388	2·8	+
	14	258	3·0	+++
	22	100	2·4	+
IV D	6	40	0·2	+
	9	40	1·0	+
	10	43	2·6	+
	18	40	2·2	+++
Normal controls		450–1400	37·5 ± 7·0**	–

* –: none present; +: small numbers, ++: moderate numbers; +++: large numbers

** Mean ± SD.

but not in the controls. This staining occurred in small isolated areas of the spinous and granular layers of the epidermis, giving a pemphigus-like appearance. T6 staining of keratinocytes was weaker than that of Langerhans cells.

In the HIV patients there was a significant correlation between the number of Langerhans cells in normal epidermis and the absolute number of T4 cells in the plasma ($r = 0{\cdot}823$; $P < 0{\cdot}001$) (Fig. 1). There was also a relationship between the number of Langerhans cells and the clinical stage of the disease.

The numbers of Langerhans cells in the epidermis and of plasma T4 lymphocytes were decreased mainly in stages III and IV of HIV infection (Fig. 2).

DISCUSSION

Our study has shown a correlation between the number of T6 positive cells (Langerhans cells) in

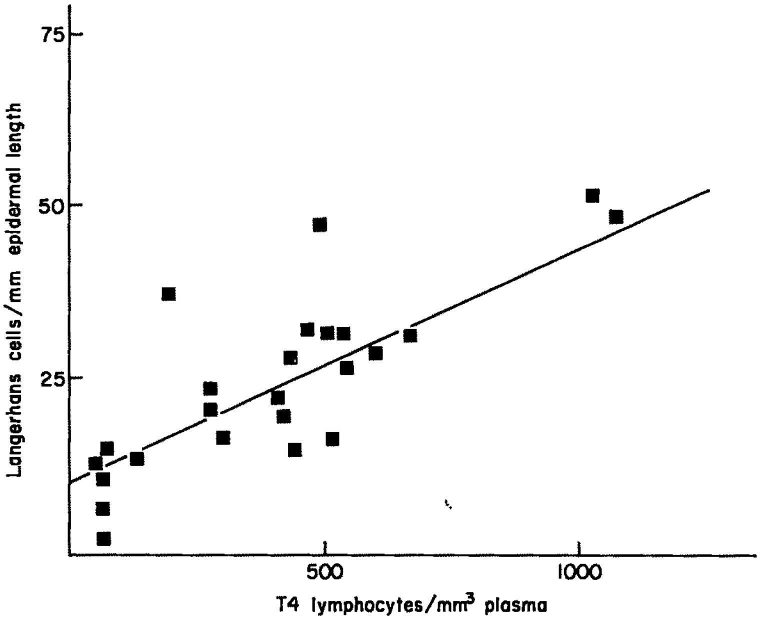

FIGURE 1. Correlation between the number of Langerhans cells in the epidermis and the numbers of T4 lymphocytes in the plasma in 24 patients with HIV infection.

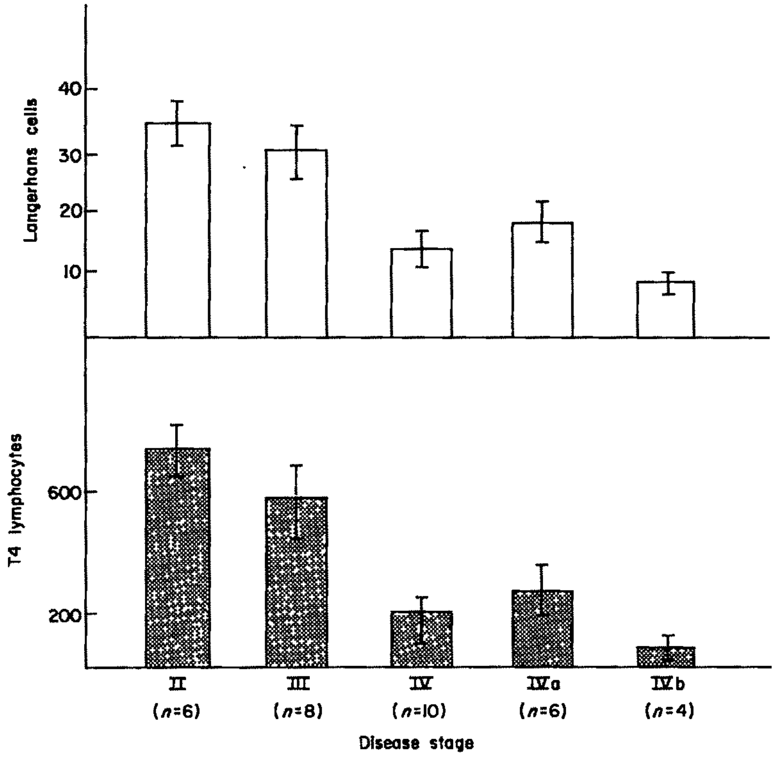

FIGURE 2. Numbers of Langerhans cells/mm epidermal length and T4 lymphocytes/mm^3 of plasma in 24 HIV patients, in relation to disease stages. Bars are means ± SD.

normal epidermis and the number of T4 lymphocytes in the plasma in patients infected with HIV. The number of Langerhans cells was related to the clinical stage of the disease, as was the number of T4 lymphocytes, and both were found to be decreased in stages III and IV. In particular, in stage IV patients T4 cells were decreased in patients with Kaposi's sarcoma.

In this study we excluded the effect of exposure to sunlight, which can lead to a decrease in Langerhans cells, by taking biopsies from the sun-protected inner side of the upper arm. None of the subjects had had recent prolonged exposure to sunlight, or PUVA or had had chemotherapy. Belsito *et al.*[6] found no decrease in the number of Langerhans cells in homosexuals and drug addicts with negative HIV serology.

The hypothesis that Langerhans cells may play an essential role in HIV infection is based on several different observations. First, HIV is preferentially replicated in cells expressing T4 membrane antigen,[1] and it has been shown that Langerhans cells express T4 antigen.[7] Secondly, HIV has recently been detected in follicular dendritic cells of lymph nodes[2] and in macrophages.[3,4] As Langerhans cells are the macrophages of the skin, they are thus candidates for early retroviral infections. Ringler,[8] demonstrated apposition of Langerhans cells and T8 cytotoxic cells in the early cutaneous manifestations of HIV infection. In confirmation of these findings, Tschachler *et al*[5] have detected HIV in Langerhans cells of patients with clinically established AIDS-related complex (ARC) or AIDS and have shown that approximately 30% of Langerhans cells display signs of damage. These authors have also shown that the cultivation of mononuclear phagocytes from a non-infected donor with a biopsy of normal skin from an AIDS patient resulted in the detection of a high level of reverse transcriptase activity in the culture supernatant.[9] Lastly, the transfer of HIV infection with a skin graft[10] seems to confirm Tschachler's studies.

Together with these results, our work suggests that Langerhans cells, in addition to T4 lymphocytes, could be one of the primary targets of HIV. If macrophages, which are the antigen presenting cells for T lymphocytes, are altered by HIV, this could account for the absence of T4 proliferation in turn leading to further T4 deficiency. Kirkpatrick[11] has provided evidence of defective antigen presentation by mononuclear cells in AIDS. A mononuclear cell preparation from a patient with AIDS did not stimulate DNA synthesis *in vitro* in T cells from the same patient or allogeneic T cells, in the presence of *Candida* antigen. However, it is possible that not all dendritic cells are altered in the same way by HIV, since interdigitating cells in the lymph nodes of AIDS patients are not decreased.[12]

What then is the means of T6 keratinocyte expression? Such expression has already been found in various skin lesions,[13,14] but not in normal skin.[15] In the HIV patients, Langerhans cells could thus be altered by HIV without the appearance of skin lesions, and such alterations could lead to fragility of the T6 antigen, with possible diffusion into the intercellular spaces. The three patients with no keratinocyte staining in our study were only at stage II of the infection, whereas those at stage IV had extensive staining possibly indicating greater alteration of Langerhans cells.

Our results suggest that macrophages play an important role in HIV infection and that numbers of Langerhans cells and T4 lymphocytes may have prognostic value in the disease.

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Plasma 5-S-cysteinyldopa as an index of melanogenesis

J.E.NIMMO*, D.J.GAWKRODGER, C.St.J.O'DOCHERTY, S.M.GOING,
I.W.PERCY-ROBB* AND J.A.A.HUNTER

Departments of *Clinical Chemistry and Dermatology, University of Edinburgh, Royal Infirmary, Edinburgh U.K.

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SUMMARY

Plasma 5-S-cysteinyldopa (5-S-CD) concentrations measured in healthy volunteers in Edinburgh, Scotland (latitude 56°N) showed only minor changes during the day. However, when measurements were performed over a 12-month period a significant rise in 5-S-CD concentration was found. Skin pigmentation and hair colour were not related to plasma 5-S-CD levels. Patients with psoriasis treated with ultraviolet-B or photochemotherapy (PUVA) developed an almost two fold increase in their plasma 5-S-CD level within the first five treatments, before pigmentation developed, subsequent increments of up to four times the pre-treatment level being found in the PUVA group. Dithranol treatment caused an increase in plasma 5-S-CD in some psoriatic patients, suggesting a possible association between skin erythema and elevated 5-S-CD levels. The value of plasma 5-S-CD in the follow-up of patients with malignant melanoma does not seem to be invalidated by unavoidable exposure of the subjects to sunlight in a temperate climate such as that of South East Scotland.

The amino acid 5-S-cysteinyldopa (5-S-CD), a precusor of phaeomelanin, is formed by the action of tyrosinase on the amino acid tyrosine. The resulting dopaquinone reacts spontaneously with cysteine *in vitro* to form 5-S-CD and probably does so *in vivo* also. This compound has been shown to be present in the urine and serum of normal healthy individuals.[1,2]

Exposure to ultraviolet radiation (UVR) is a powerful stimulus to melanin synthesis and both the urinary excretion and the serum concentration of 5-S-CD are known to increase in response to natural sunlight and artifical UVR.[3–7]

Malignant melanoma is usually a pigmented tumour and in patients with secondary spread of the neoplasm the urinary excretion of 5-S-CD may be increased.[8] There is evidence that plasma 5-S-CD concentrations may also be raised in patients with metastatic malignant melanoma.[2]

Plasma 5-S-CD may, therefore, be useful as a tumour marker for malignant melanoma. Therefore, in the present study we aimed to establish the normal pattern of variation in plasma

Correspondence: Professor I.W. Percy-Robb, Department of Pathological Biochemistry, Western Infirmary, Glasgow G11 6NT, U.K.

5-S-CD concentrations in healthy individuals through the day, and to examine the effect of exposure to natural sunlight in the U.K. and to measured amounts of UVR, on plasma 5-S-CD levels. In addition we studied the relationship between skin and hair colour and plasma levels of 5-S-CD.

METHODS

Healthy volunteers

Nine normal healthy Caucasian volunteers (four males, five females; age range 24 to 47 years) participated in the study of seasonal variation in plasma 5-S-CD levels. Blood samples were taken from these subjects at monthly intervals over a period of 1 year. Four of these volunteers also took part in a study of the diurnal variation in plasma 5-S-CD. Blood samples were taken at hourly intervals over a period of 12 h (0900h to 2100h). The subjects fasted from the previous evening until after the first sample was taken.

Eight Caucasian volunteers with red hair and ten volunteers of Afro-Carribean ethnic origin (black skinned), resident in Edinburgh and Birmingham respectively, had blood samples taken for measurement of plasma 5-S-CD.

Psoriasis patients

Seven patients receiving short wavelength UVR (UVB : 280–315 nm) and seven patients receiving combined psoralen and long wavelength UVR (PUVA : 320–400 nm) had blood samples taken before starting treatment and at weekly intervals throughout the course of therapy in order to examine the effects of UVR on plasma 5-S-CD concentrations. Patients receiving PUVA therapy were commenced on a dose of 1–2 J/cm^2, which was increased gradually, by 0·5–1 J/cm^2 per session, to a maximum of 9·5 J/cm^2. Patients on UVB therapy were commenced on a dose of approximately 0·15 J/cm^2, and this was increased by 0·05–0·2 J/cm^2 per session, to a maximum dose of 1·35 J/cm^2. The degree of pigmentation and erythema induced by the treatment was recorded.

The possibility that cutaneous cell damage itself could cause a rise in plasma 5-S-CD was investigated in psoriatic patients receiving monotherapy with dithranol, which induces an irritant erythema and staining of the skin. Dithranol in Lassar's paste was started at a concentration of 0·1%, and gradually increased to 0·5% or 1% depending on the patient's response. Six psoriatic patients, four males and two females, aged between 23 and 72 years (mean 50 years), were studied. Blood samples were taken before treatment commenced, and at approximately 7-day intervals over a 3-week period. The onset and severity of erythema was recorded.

Sample collection and analysis

Venous blood samples were collected in lithium-heparin tubes containing dithiothreitol at a final concentration of 5 mmol/l. The plasma was separated by centrifugation at 2000 *g* for 10 min, at ambient temperature, and 2 ml of plasma was used for each analysis. Specimens were stored at −20°C. The plasma samples were assayed using 5-S-D-cysteinyl-L-dopa, prepared according to the method of Agrup *et al.*,[9] as the internal standard. Plasma containing internal standard was placed in a tube to which 50 μl 0·3 mol/l EDTA, 50 μl 50 mmol/l reduced glutathione, and 20 mg activated alumina were added. The contents of the tube were mixed, and 200 μl 1 mol/l Tris buffer (pH 8·6) was added during mixing. The tube was shaken vigorously for 15 min, centrifuged at 3000 *g* for 5 min, and the supernatant discarded. The alumina was washed three times with 3 mmol/l EDTA, (pH 8·0) and the tube centrifuged at 3000 *g* for 5 min after the final wash. The 5-S-CD was eluted with 150 μl 0·2 mol/l $HClO_4$, and 50 μl of eluate was used in the determination of 5-S-CD concentration by high pressure liquid chromatography (HPLC).

The HPLC system consisted of an Altex 110A pump (Altex Scientific Inc., Berkeley, California, U.S.A.), Rheodyne injection valve, model 7125 (Rheodyne, Cotati, California, U.S.A.) equipped with a 50 μl injection loop, and a 250 × 4·6 mm stainless steel column packed with chemically bonded 5 μm C18 material (Hypersil ODS, Shandon Southern Products Ltd., Runcorn, Cheshire, U.K.). Detection was by a model LC-10 amperometric detector (Bioanalytical Systems Inc., West Lafayette, Indiana, U.S.A.). The detector potential was set at 0·7 V against the Ag/AgCl reference electrode. The working electrode was glassy carbon. The amounts of 5-S-CD were estimated by the ratios of the peak heights.

The mobile phase contained methanesulphonic acid (6·0 g/l) and orthophosphoric acid (3·0 g/l) in purified water (Elgastat Spectrum reverse osmosis system, Elga Group, Buckinghamshire, U.K.). The pH of the mobile phase was 2·5.

Alumina extraction resulted in the production of a solution which on HPLC analysis showed good separation of D-and L-isomers of 5-S-CD from one another and from other solutes detected by electrochemical means (the D-isomer elution time was approximately 8 min; the L-isomer elution time was 10 min).

Evidence for the identity of the plasma peak as L-5- S- CD was that the substance was found to co-chromatograph exactly with authentic L-5-S-CD under varying conditions of pH and concentration of ion-pairing agent, and that the oxidation properties of this substance were similar to those of the authentic L-5-S-CD.

RESULTS

The mean plasma 5-S-CD levels in four subjects over a 12 h period are shown in Figure 1. There was some variation in mean 5-S-CD concentration during the day, the lowest value being 4·6 nmol/l at 1300h with peaks of 6·3 nmol/l at 1600 h and 2100 h. Plasma 5-S-CD rose in the mid

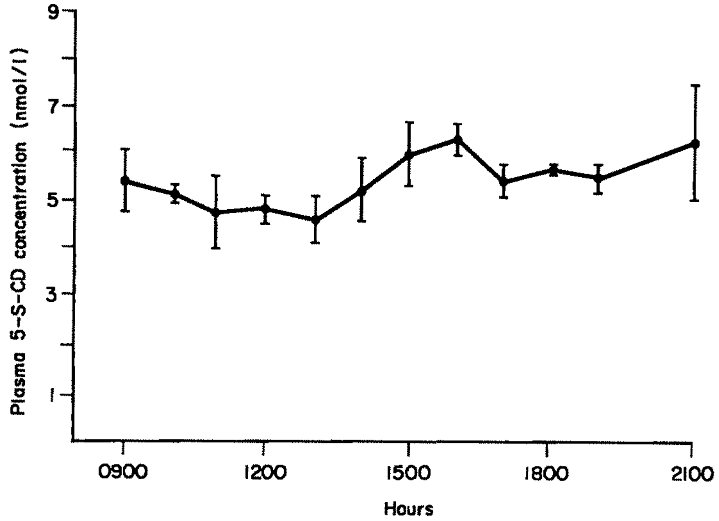

FIGURE 1. Plasma 5-S-CD concentrations in four normal subjects over a 12 h period. Points are means ± SD.

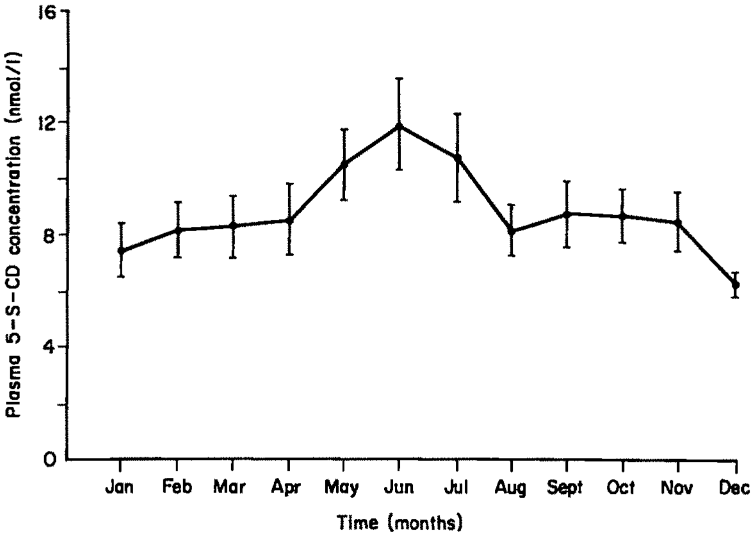

FIGURE 2. Plasma 5-S-CD levels in 11 normal volunteers over a 12-month period. Points are means ± SEM.

afternoon and in the evening, possibly related to the eating of meals. The difference in mean 5-S-CD levels between 1300 h and 2100 h was not statistically significant ($P > 0{\cdot}05$; paired *t*-test), suggesting that in further studies the time of day at which sampling is performed should not be a critical factor.

The seasonal variation in mean plasma 5-S-CD levels is shown in Figure 2. Plasma 5-S-CD levels began to rise during the spring months, reaching a peak in June, and falling thereafter,

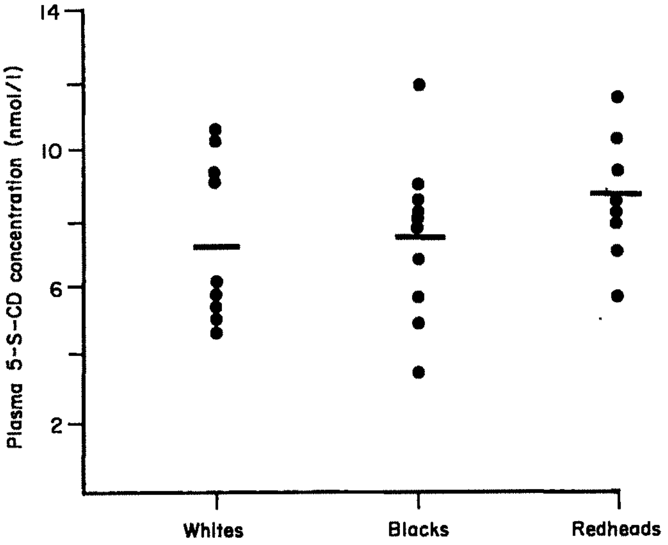

FIGURE 3. Plasma 5-S-CD values in normal volunteers with different skin and hair colour. Bars indicate means.

TABLE 1. Plasma 5-S-CD concentrations (nmol/l) in patients receiving UVB therapy (percentage of pre-treatment value shown in parenthesis)

Subject no.	Skin type	Total dose (J/cm^2)	Pre-treatment	No. of treatments														
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
1	II	7·1	7·8	17·4 (223)	...	15·1 (194)	...	16·1 (206)	...	8·8 (113)	...	5·8 (74)	...	5·5 (71)	...	...	...	8·2 (105)
2	II	4·5	10·0	...	...	17·3 (173)	...	...	10·5 (105)	...	...	7·2 (72)	...	...	...	...	9·7 (97)	...
3	III	1·9	11·2	...	16·8 (147)	27·1 (242)	13·7 (122)	...	11·6 (104)	11·0 (98)	7·9 (71)	...	9·7 (87)	...	...	...	...	...
4	III	5·9	14·5	...	18·0 (124)	20·5 (141)	...	23·1 (159)	14·8 (102)	...	17·9 (123)	17·5 (121)	...	12·9 (89)	13·4 (92)	...	...	...
5	III	6·2	15·2	...	21·8 (143)	37·7 (248)	...	28·2 (186)	28·1 (185)	...	22·8 (150)	23·0 (151)	...	21·8 (143)	...	...	...	...
6	IV	6·5	8·0	14·1 (176)	...	...	12·8 (160)	...	17·3 (216)	4·6 (58)	...	...	...	7·9 (99)	...	7·9 (99)	...	...
7	IV	3·4	12·2	28·6 (234)	...	27·8 (228)	...	...	18·9 (155)	...	...	...	...	10·2 (84)	19·3 (158)	...	...	...

TABLE 2. Plasma 5-S-CD concentrations in patients receiving PUVA therapy (percentage of pre-treatment value shown in parenthesis)

Subject no.	Skin type	Total dose (J/cm²)	Pre-treatment	No. of treatments															
				1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
1	I	159·0	7·6	14·5 (191)	…	36·9 (486)	…	29·9 (393)	…	16·4 (216)	…	10·8 (142)	10·9 (143)	8·7 (114)	9·6 (126)	9·1 (120)	16·6 (218)	12·8 (168)	8·7 (114)
2	II	61·5	10·6	10·3 (97)	…	8·3 (78)	…	…	20·1 (190)	23·9 (225)	5·3 (50)	…	17·3 (163)	…	10·9 (103)	…	…	…	…
3	II	104·5	9·5	…	18·0 (189)	…	20·0 (211)	14·7 (155)	12·5 (132)	12·0 (126)	…	…	…	14·8 (156)	9·1 (96)	13·5 (142)	…	…	…
4	II	69·0	3·6	3·2 (89)	…	6·3 (175)	…	14·6 (406)	…	4·9 (135)	9·2 (255)	4·3 (119)	…	10·8 (300)	…	15·3 (425)	…	8·4 (233)	…
5	III	58·5	6·3	17·1 (271)	…	16·7 (265)	…	17·6 (279)	…	13·8 (219)	…	15·8 (251)	…	…	17·6 (279)	14·8 (235)	11·6 (184)	…	11·6 (184)
6	IV	119·5	11·3	…	14·6 (129)	…	11·6 (103)	…	14·0 (124)	…	…	21·4 (189)	…	13·2 (117)	…	…	…	…	…
7	IV	71·3	10·7	…	…	…	11·7 (109)	…	15·1 (141)	20·4 (191)	…	…	14·8 (138)	…	…	…	…	…	…

with the lowest levels occurring in December. Results varied considerably between individuals. The mean level in June was significantly higher than the mean in December ($P < 0{\cdot}01$; paired *t*-test).

The plasma 5-S-CD levels in volunteers with different hair and skin pigmentation are shown in Figure 3. There were no significant differences in the plasma 5-S-CD concentrations between any two of the three groups ($P > 0{\cdot}05$; unpaired *t*-test).

Results from psoriasis patients undergoing UVR therapy are given in Tables 1 and 2. Plasma 5-S-CD levels in all patients receiving PUVA began to increase after the first 2 to 5 treatments, reaching peak values which were between 2 and 5 times the pre-treatment level. The pattern of change in 5-S-CD levels varied considerably between individuals. In some patients the 5-S-CD level following the initial rise fell to approximately the pre-treatment value, but in others the initial increase was followed by fluctuations in the 5-S-CD concentration throughout treatment. However, the initial peak in 5-S-CD concentration was a consistent finding occurring in most patients between 10 and 20 days after starting PUVA.

UVB therapy also resulted in a rise in plasma 5-S-CD levels which occurred shortly after commencing treatment, with maximum values of between $1\frac{1}{2}$ and $2\frac{1}{2}$ times pre-treatment levels. Patients receiving UVB showed less individual variability in 5-S-CD levels than those who were receiving PUVA. In general, the trend in patients receiving PUVA and UVB, was for an initial rise in 5-S-CD levels, occurring earlier in PUVA patients, followed by a return towards pre-treatment values.

The topical application of dithranol produced a rise in the plasma 5-S-CD in three of the six subjects at about the 7th day after starting treatment. However, the group mean 5-S-CD concentration, between the 6th and 10th day of therapy, was not significantly different from the mean starting level (unpaired *t*-test). In the three patients who did show a rise in 5-S-CD levels, no relationship was found between this and the onset of dithranol-induced erythema.

There was no evidence that 5-S-CD was generated from dopa-quinone by the assay procedure, although this remains a possible source of error.

DISCUSSION

This study has demonstrated the effect of various factors on plasma 5-S-CD concentrations. We have shown small, but statistically significant changes in 5-S-CD levels during the day, and larger changes in 5-S-CD levels during the year. Patients receiving treatment with UVR had dramatic changes in their plasma 5-S-CD levels although an individual's skin and hair colour were not related to the plasma 5-S-CD level.

When setting up a new assay it is important to determine whether there are extraneous factors which might modify the results. For example, UVR exposure could limit the usefulness of a plasma 5-S-CD assay in patients with malignant melanoma, by producing false positive results. Exposure to natural sunlight in Edinburgh (latitude 56°N) was associated with a significant increase in the plasma 5-S-CD levels of normal volunteers from 6·3 nmol/l in December to 11·9 nmol/l in June, a virtual doubling from winter to summer.

The effect of UVB and PUVA on plasma 5-S-CD levels was demonstrated in psoriasis patients undergoing treatment with these modalities. In each group a rise in plasma 5-S-CD was seen shortly after starting treatment, although the peak values for the UVB patients occurred earlier and were smaller in comparison with pre-treatment levels, than those in the PUVA group. One might expect individuals whose skin became more pigmented to have higher levels of 5-S-CD, but it was difficult to relate the changes in plasma 5-S-CD levels to the production of erythema or tanning. However, the initial rise in plasma 5-S-CD levels in both PUVA and UVB patients occurred before tanning was observed although erythema was often present, particularly in patients with skin types I (always burn, never tan) and II (sometimes burn, eventually tan), suggesting a possible association between an increase in 5-S-CD and skin inflammation or damage.

Alternatively, the early pre-tanning rise in 5-S-CD levels may have resulted from stimulation of melanin production by UVR, with intermediates such as 5-S-CD being released into the bloodstream before pigmentation became apparent. In fact, the induction of tyrosinase in melanocytes by UVR has been demonstrated.[10]

Topical dithranol therapy, with its associated erythema and staining, produced a rise in the plasma 5-S-CD level in three out of six patients, but we were unable to demonstrate a relationship between the appearance of the erythema and the changes in plasma 5-S-CD levels. The mechanisms of these changes remain to be explained, although if the patients were stressed and blood catecholamine levels were high, spontaneous oxidation of the catecholamines and their resulting quinones could be a source of cysteinyldopamines, which may be responsible for the 5-S-CD-like activity detected.

Increases in the plasma 5-S-CD concentration in psoriatic patients have already been reported to coincide with episodes of erythema,[6,7] and such changes may be more closely linked to cell damage than to stimulation of melanin production in the melanocytes. On the other hand, our observation that the UVR induced increases in 5-S-CD levels were of the same magnitude in individuals with skin types III and IV as in those with skin types I and II, suggests that skin damage is not the only factor involved, since patients with skin types III and IV often showed no sign of erythema during treatment.

No difference in 5-S-CD values was found between Caucasian subjects with red hair and Caucasian subjects with blond or brown hair, indicating that a predominance of phaeomelanin in the red haired group was not associated with higher levels of phaeomelanin precursors in the plasma. Likewise the 5-S-CD concentrations in black skinned subjects were similar to those in Caucasian controls. Although there is no disparity in the melanocyte numbers between blacks and whites, the pigmentary distinction being mainly due to differences in the distribution and breakdown of melanosomes, subjects with black skin do produce more melanin, although the breakdown of it is less than in Caucasians.

Sunlight can increase the urinary levels of 5-S-CD and this is important when considering the value of this assay in detecting metastatic malignant melanoma. Nixon[4] found that fluctuations in urinary 5-S-CD in healthy subjects in Australia were so great that measurement was useless in that very sunny climate, unless patients avoided all sunlight for several weeks prior to the 5-S-CD estimation. Rorsman *et al.*[3] concluded that measurement of urinary 5-S-CD was not helpful during the summer in Sweden due to the increase in 5-S-CD output during these months, seen in both normal healthy controls and malignant melanoma patients with no evidence of metastatic disease. The increase in plasma 5-S-CD levels which we observed during the summer months, although significant, was not as great as the increase in urinary output of 5-S-CD described by Rorsman *et al.*[3] We have adopted 20 nmol/l as the upper limit of our reference range for the detection of metastatic disease in malignant melanoma patients as, even in summer, the 5-S-CD level in healthy controls did not exceed this figure. Nevertheless, UVR can have an effect on plasma 5-S-CD and it is important to know the amount of sun exposure a patient has had. Slightly raised 5-S-CD levels must be interpreted with care, particularly during the summer months.

Thus we have demonstrated that exposure to both natural and therapeutic UVR induces fluctuations in plasma 5-S-CD levels. However, we have found that the amount of sunshine encountered in Edinburgh caused only minor changes in 5-S-CD which we feel are insufficient to invalidate the use of this assay in detecting metastatic malignant melanoma, even during the summer months.

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A closer look at *Pthirus pubis*

D.A.BURNS AND T.A.SIMS
Leicester Royal Infirmary, Leicester, U.K.

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Pthirus pubis, commonly referred to as the pubic louse or crab louse, and delicately designated papillon d'amour by the French, is an insect whose biology has been rather neglected in comparison with the attention lavished on the head louse and clothing louse. Even its name is frequently misspelled. The generic name was intended to be Phthirus, but as a result of a misprint, Pthirus was confirmed as the official name by the International Commission on Zoological Nomenclature.[1] It has no close relatives other than a similar species found on gorillas.

We present here a number of scanning electron micrographs which demonstrate some of the anatomical features of this parasite. The structure of the mouthparts is demonstrated, and the mechanism of feeding described.

Anatomically *Pthirus pubis* is quite distinct from *Pediculus*, having a short, squat body flattened dorsoventrally and the abdomen apparently compressed longitudinally so that the first three abdominal spiracles are almost in a transverse line. One pair of thoracic spiracles (Fig. 1) and six pairs of abdominal spiracles constitute the external openings of the respiratory system, an elaborate arrangement of air tubes or tracheae which ramify throughout the insect's organs (Fig. 2).

The first pair of legs are slender, with a fine claw, but the second and third pairs of legs carry heavy, pincer-like claws (Fig. 3) adapted for grasping hair. These heavy claws cling to hair so firmly that the attempted removal of a crab louse from the skin often results in severe damage to the legs. When removed from hair *Pthirus pubis* is almost helpless, whereas *Pediculus* moves around quite readily. On the host, *Pediculus* wanders extensively, whereas *Pthirus* is relatively sedentary. Nuttall[2] raised two crab lice experimentally, and plotted their movements on the skin. Neither of them wandered beyond the borders of a rectangle of skin measuring 16 × 10 cm.

The head carries a pair of five-segmented antennae. In work on the clothing louse, Wigglesworth[3] demonstrated that the antennae are responsible for odour and humidity detection, and that the so-called 'peg organ' at the tip of the antenna was the seat of the sense of smell. Scanning electron microscopy of the tip of the fifth antennal segment of *Pthirus* shows a group of protrusions, some with pointed tips, others with rounded tips, which constitute the peg organ (Fig. 4). Just posterior to the antennae are a pair of simple eyes.

Pthirus pubis is a member of the Order Anoplura or 'sucking lice'. It is a solenophage[4] (vessel feeder—from the Greek 'pipe' + 'eating'), introducing its mouthparts directly into a blood

Correspondence: Dr D.A.Burns, Leicester Royal Infirmary, Leicester LE1 5WW, U.K.

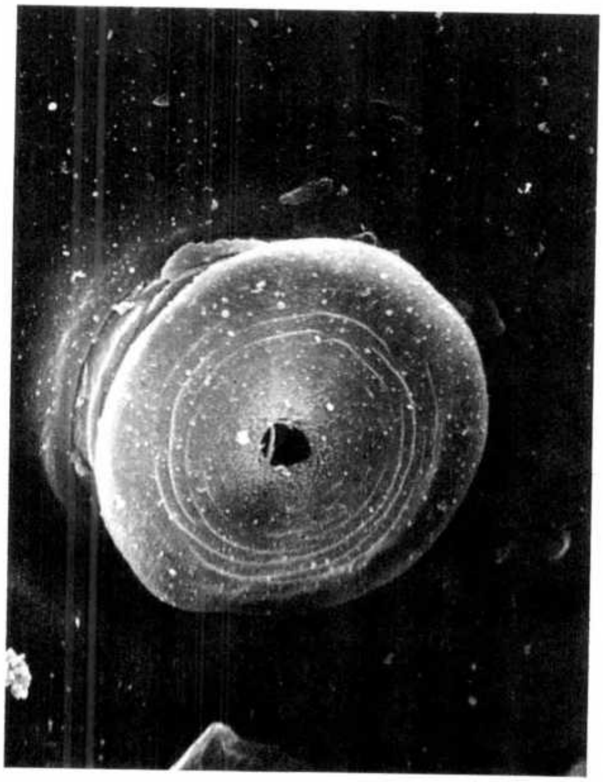

FIGURE 1. Scanning electron micrograph of a thoracic spiracle. (Original × 1500).

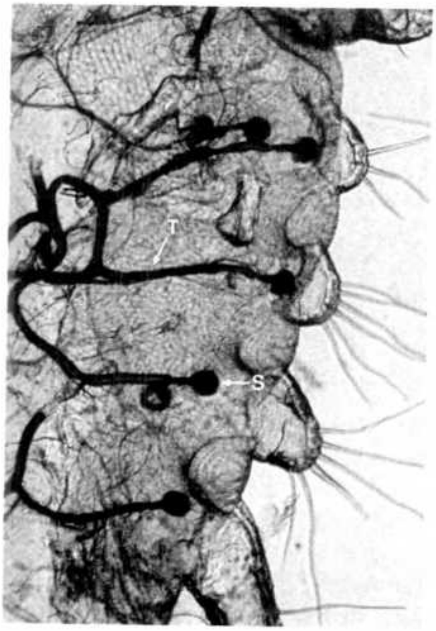

FIGURE 2. Photomicrograph showing abdominal spiracles (S) and tracheal system (T). (Original × 100).

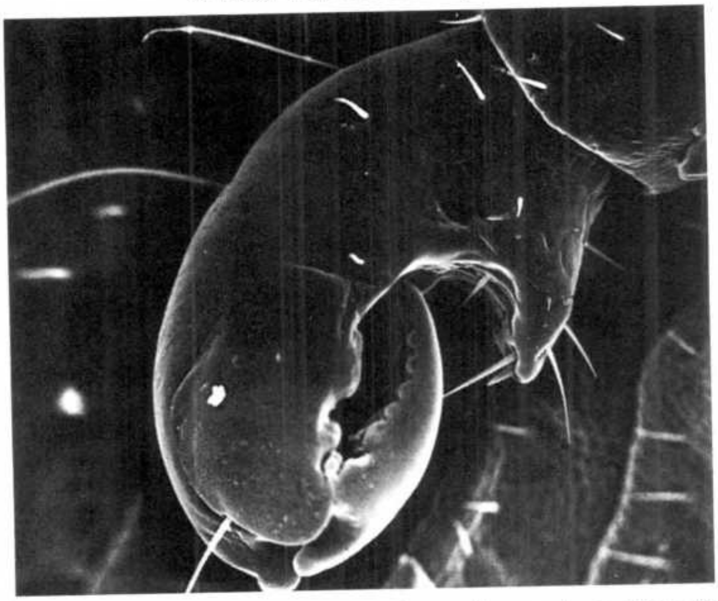

FIGURE 3. Scanning electron micrograph of the tarsal claw on the second pair of legs. (Original × 200).

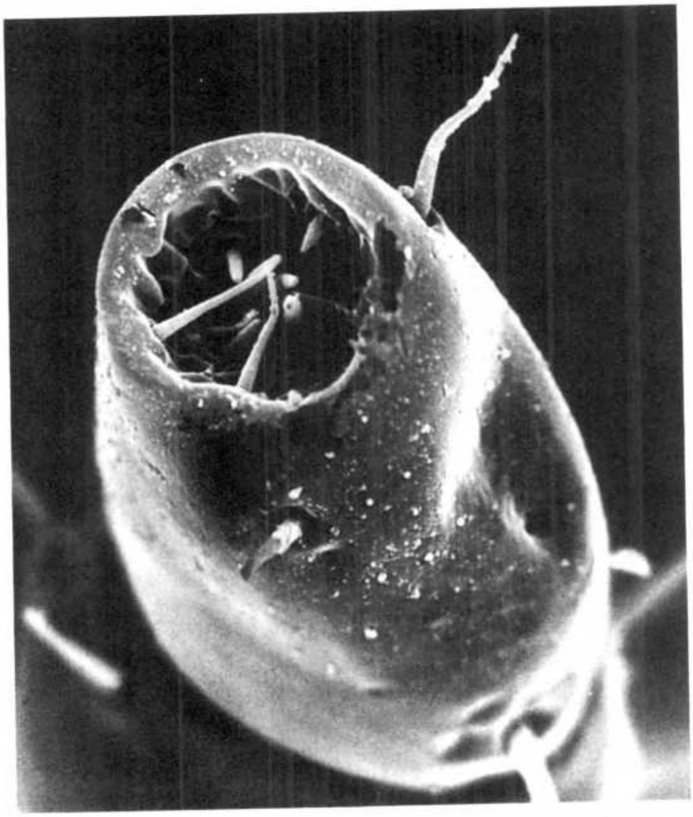

FIGURE 4. Scanning electron micrograph of the tip of an antenna showing the peg organ. (Original × 1000).

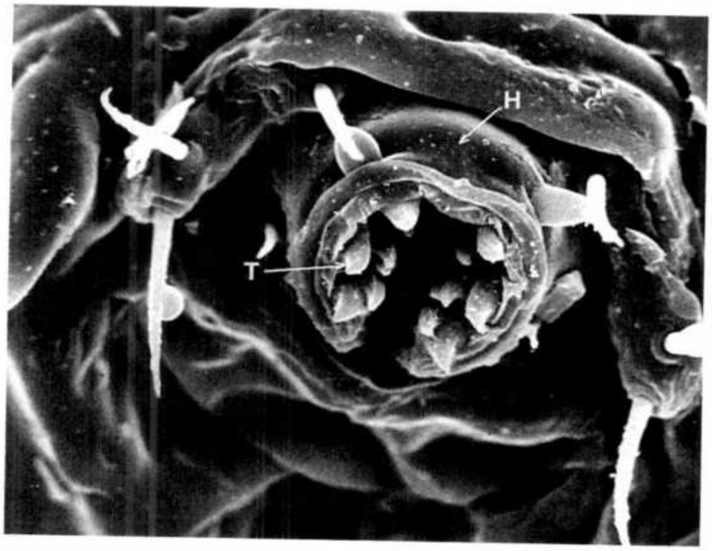

FIGURE 5. Mouthparts. The haustellum (H) is retracted. The buccal teeth (T) are clearly visible. (Original × 1000).

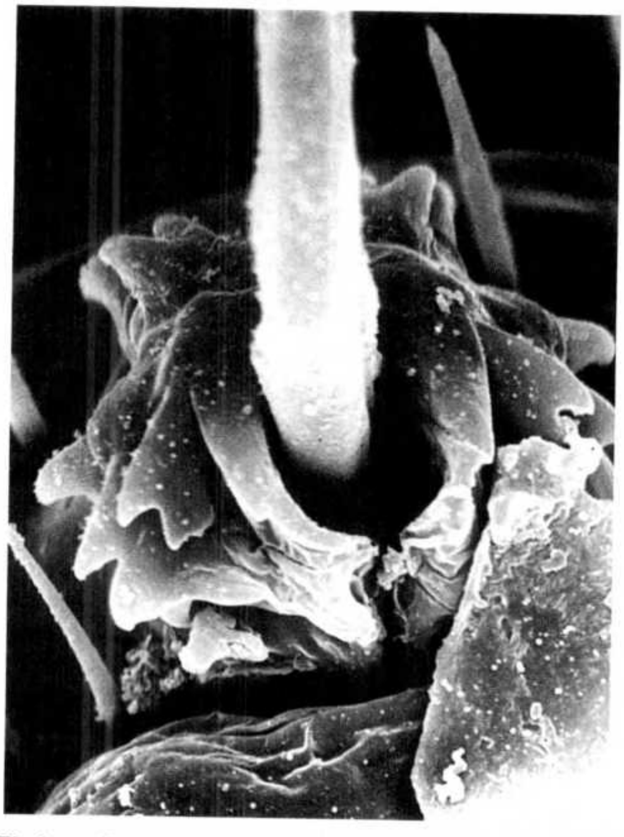

FIGURE 6. The haustellum is protruded and the buccal teeth everted. (Original × 1500).

vessel to withdraw blood. The components of the mouthparts responsible for probing the skin and piercing a blood vessel are a group of stylets which are kept withdrawn within the head unless the insect is feeding. Lavoipierre[5] observed the feeding mechanism of an anopluran louse on a transilluminated mouse ear. In the front of the head is a small, snout-like tube, the haustellum, which is soft, eversible, and armed with teeth. Figure 5 shows the haustellum retracted, and the buccal teeth are clearly visible. When the louse is about to feed the haustellum is everted, and the buccal teeth rotated outwards. The teeth cut into the epidermis with a movement compared by Lavoipierre[5] to that of a rotary saw, and the haustellum is gradually driven into the dermis. It eventually comes to rest with the buccal teeth fully everted, anchoring the mouthparts in the skin (Fig. 6). Once fixed in the skin, a bundle of stylets (dorsal, median and ventral) are pushed forward through the opening in the haustellum by protractor muscles within

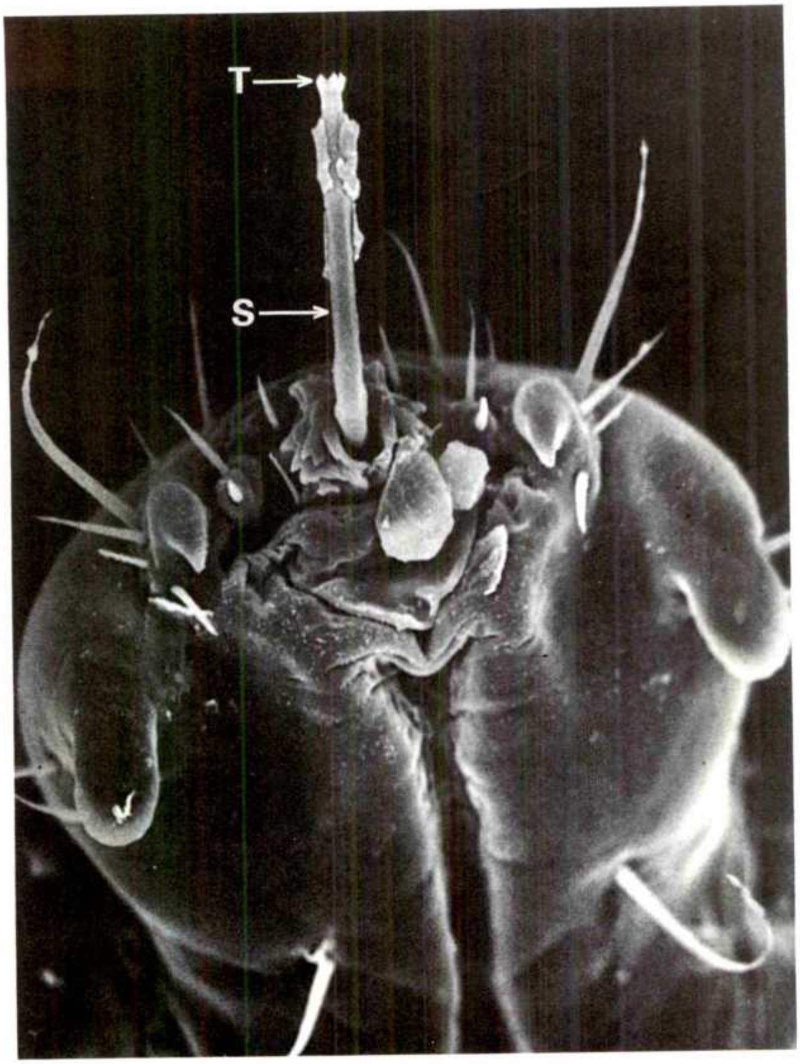

FIGURE 7. The stylet bundle (S) is protruded. Small teeth (T) are visible on the tip of the stylet bundle. (Original × 300).

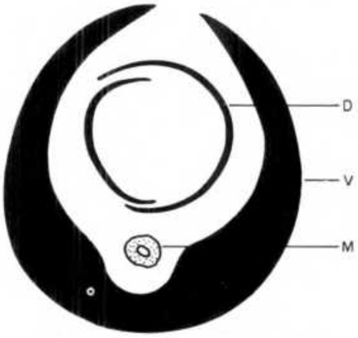

FIGURE 8. Diagrammatic cross-section of the arrangement of the stylet components. Dorsal stylets (D), median stylet (M), ventral stylet (V).

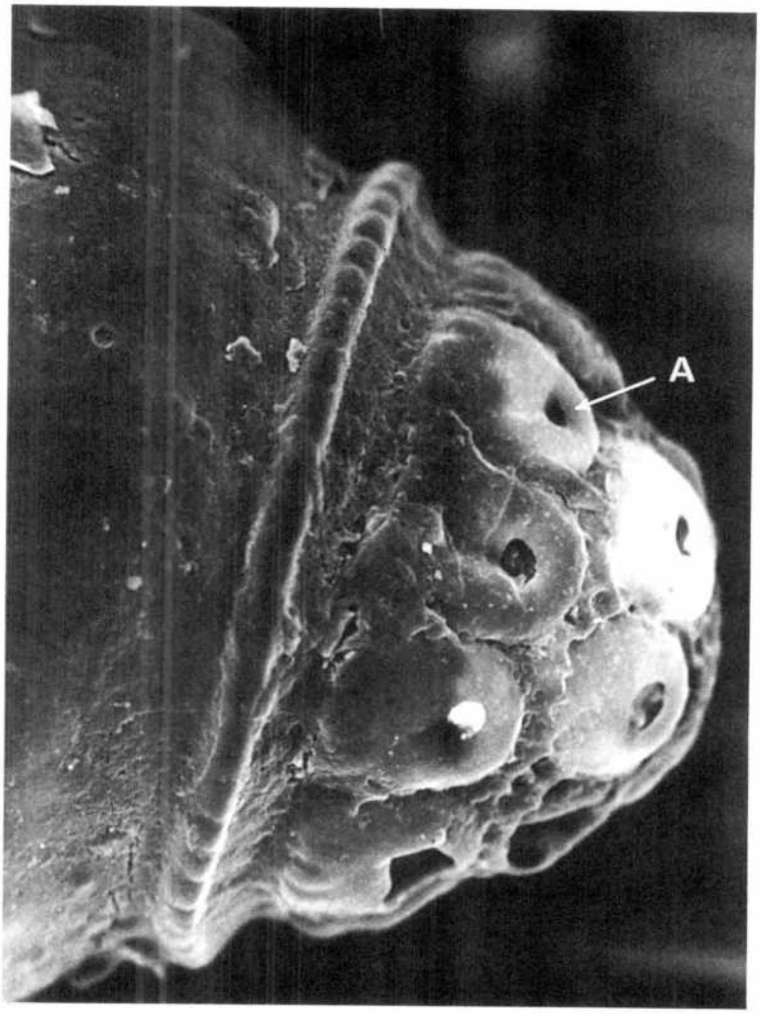

FIGURE 9. Aeropyles (A) in the operculum of an egg. (Original × 300).

the head of the louse. The stylets are advanced into the dermis as a single bundle or fascicle, and probe for a small blood vessel. Once the stylet bundle has pierced a blood vessel feeding begins. The protruded stylet bundle is clearly seen in Figure 7. The stylet bundle is composed of two dorsal stylets, closely apposed to form a hollow tube. This tube constitutes the food canal,[6] and leads into the pharynx. The median stylet encloses a salivary canal, and the ventral stylet embraces the other stylets, holding them together as a fascicle (Fig. 8). The tip of the ventral stylet is armed with small teeth which help penetration and probing of the dermis (Fig. 7).

The eggs of the crab louse, like those of the head louse, are cemented to hair shafts. A cap (operculum) covers the free end of the egg, and during the hatching process this cap is pushed off like a manhole-cover by the emerging louse. The operculum contains a number of air cavities known as aeropyles, the openings of which are clearly visible in Figure 9. When the louse is ready to emerge from the egg it pushes its mouthparts through the membrane which encloses it, into an air space in the egg cap. It then swallows air which passes through its gut and collects behind its rear end, propelling it outwards on a cushion of compressed air.

The crab louse, if not a thing of beauty except to an interested minority, is certainly beautifully equipped for its mode of life. It deserves admiration, however grudgingly bestowed, for its remarkable adaptation to a hostile environment, and warrants further study.

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Comment

Self help groups for patients with chronic skin diseases

R.A.LOGAN

St. Johns Hospital for Diseases of the Skin, 5 Lisle Street, London WC2H 7BJ, U.K.

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Patients with chronic skin diseases may experience feelings of shame or guilt concerning their skin. They might be ostracized at school, work or in recreation. They could be discriminated against in the job market. They might feel sexually unattractive. Patients do not like to talk about these intimate details in the context of a short hospital consultation, possibly because they feel that they would be wasting the doctor's time. Nevertheless, these psychosocial aspects of skin diseases can seriously damage a patient's self-esteem. Their ability to cope with even minor disease will fall, leading to repeated and frequent visits to the dermatologist, ostensibly because 'the treatment isn't working'.

We all recognize this stereotype of patient behaviour, and yet it is difficult to feel anything but irritation when the patient's name appears once more on the clinic list. Attempts at discharging the patient are usually in vain. There follows an unrewarding series of encounters in which both the full range of treatment options, and the patience of the doctor, is rapidly exhausted without apparent improvement in the patient's condition.

A failure to address this important side of patient management is one of the points raised by Jowett and Ryan[1] who drew to our attention some of the ways in which we, as dermatologists, fall short of delivering ideal care to our patients. Similar views were expressed by the chairman of the Psoriasis Association following the screening on BBC Television of *The Singing Detective*.[2]

Much of our patients' dissatisfaction stems from the fact that, in most NHS practice, there is insufficient time available for us to address adequately the psychosocial aspects of disease as well as provide suitable 'physical' treatment. To some extent these shortcomings are alleviated in the private sector, where more time is available for fuller discussion. However, there is a limit to how much information can be absorbed by even the most receptive patient during one visit to the doctor. Reinforcement and clarification may be aided by the use of patient information leaflets or books, but these are impersonal. There is still a need for our patients to discuss their own feelings and share their experiences with other 'sufferers'.

One possible solution to this deficiency in our present health care system is through group therapy. I have recently returned from the first 'International Training Workshop on group-orientated patient education, health promotion and coping with chronic disease'. This course had special reference to psoriasis and was held in Maastricht, Holland, 13–18 September 1987, as a joint project of the WHO/EURO Health Promotion Unit, the Dutch Ministry of Welfare and the Institute of Self-Care Research (affiliated to the University of Limburg, Holland). Participants came from Holland, Belgium, West Germany, the U.K., Sweden, Yugoslavia and the U.S.A. During the course we were introduced to a novel concept of group therapy which may prove valuable as an extra dimension in the management of many chronic diseases, including skin disorders.

This concept has been given the enigmatic title: 'Duo-formula group therapy'. This means that groups of approximately seven patients (e.g. with psoriasis) are brought together for a series of eight to ten two-hour meetings on a weekly basis. The group is lead by a balanced pair (duo) working in partnership. The pair will normally consist of a physician (known as a co-worker) and an experienced patient (the co-helper). The physician provides the professional expertise, and the co-helper shares his or her personal experience of both the physical and the emotional aspects of the disease in question. Ideally the leading pair should have trained together in the group therapy technique. They are there not to dominate the discussion, but in a guiding role.

During the series of meetings a programme of topics for discussion is followed. These are chosen to provide an opportunity for patients to talk about many aspects of their disease ranging from the safe and tangible (e.g. the use of topical medication) to such personal and emotive subjects as coping with shame, shyness and sexual difficulties. The structure of the meetings may be modified to include time for activities such as relaxation therapy or art therapy, depending upon the requirements of the group. Meetings end with a short discussion on the group process itself to identify any possible flash-points, such as excessive dominance or aggression within the group. Between meetings the participants are asked to record their daily activities in a 'health journal' which is designed to provide an objective measure of changes in behaviour patterns relating to the disease in question.

The aim of these meetings is to improve the quality of life of patients with chronic diseases such as psoriasis, and to increase their ability to look after their own health. The meetings provide a forum for patient education, mutual support, improvement in self-care ability, and an overall reduction in dependent 'illness behaviour'. The effect of group therapy on these criteria can be measured using specially designed self-reporting questionnaires and a series of interviews. Beneficial effects have been recorded both for psoriasis[3] and in other clinical situations such as post-myocardial infarction syndrome.[4]

The meetings are also highly illuminating for the physician. They demonstrate the misconceptions, subterfuges and emotions of patients, which can remain like dust under the rug in the course of conventional clinical consultation.

The use of group therapy appears to be much greater in Eastern Block countries such as Czechoslovakia than in the West (Katja Beaman, personal communication, 1987). Perhaps this is because it is cheaper to deliver than conventional health care. However, there does appear to be a growing interest in patient participation groups and self-care in the U.K., particularly amongst general practitioners.[5,6] Getting our patients to look after their own disease is not only better for the patient, but it can also have important spin-offs in these days of financial constraints on the NHS. Devolution of health care away from hospital and into the home dramatically cuts costs. For example, it has been estimated (Professor G. Swanbeck, personal communication, 1987) that the comparative costs of caring for a patient with moderately severe psoriasis for 1 year would be something like this:

Hospital admission × 2	18,000 Dutch Guilders
Canary Islands × 2	7,000 Dutch Guilders
Treatment in day care centre	1,500 Dutch Guilders
Home treatment (self-care)	200 Dutch Guilders

As the financial squeeze tightens, fewer beds will be at our disposal. The 'Day Care Centre' will become a more common phenomenon. This would be an ideal location for group activities, and architectural planning should provide suitable space for this.

Setting up group therapy will not be everyone's cup of tea. It is time-consuming and labour intensive. Deciding which patients to involve will demand care and only a proportion of patients will be suitable and motivated to attend. Patient support groups such as the Psoriasis Association may be invaluable as a source of recruitment. In due course it should be possible to involve other interested professionals, such as the newly emerging nurse specialists, in the running of the group. However, it appears that if the doctor later tries to disappear completely from the group, this will breed resentment among the participants.[5]

The Institute of Self-Care Research is acting as a WHO Collaborating Centre in the field of self-care and health promotion. Further information and a consultative service is available from: Dr M. Bremer Schulte, Director, Institute for Self-Care Research, Boschstraat 45, 6211 AT Maastricht, Holland. Tel: 31(0)43 215423.

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Permeation of nickel through human skin *in vitro*—effect of vehicles

ANN FULLERTON, J.R.ANDERSEN AND ANNIE HOELGAARD

Department of Pharmaceutics and Chemistry, Royal Danish School of Pharmacy, 2 Universitetsparken, DK-2100 Copenhagen, Denmark.

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SUMMARY

The effect of the vehicle on the permeation rate of nickel ions through excised human skin was evaluated. Different hydrogels were compared with the standard patch test in petrolatum. A hydroxypropyl methylcellulose gel seemed to be the most promising alternative to petrolatum. It gave high bioavailability of the nickel and had good film forming properties leaving the nickel spread across the skin surface as a thin film without microscopically detectable crystals. The content of nickel in the various skin layers after cutaneous application was determined, and nickel was found to accumulate in the epidermis, probably due to epidermal binding. A significant amount of nickel was found also in the dermis. Occlusion and application of higher nickel concentrations increased the transport rate and must be considered in patch testing using this hydrogel. We conclude that nickel permeation is highly dependent of the choice of vehicle and the vehicle should, therefore, be an important consideration in patch testing with nickel.

False negative and irritant reactions may occur with the normal patch test procedure for nickel allergy using 5% $NiSO_4$ in petrolatum. The influence exerted by the vehicle has not been adequately investigated, but is an important consideration in patch testing.

False negative reactions are difficult to identify, but may be due to failure of the vehicle to release the test substance and thus insufficient penetration of the allergen. False positive reactions are usually irritant reactions and may be due to uneven dispersion of nickel as crystals of varying size in the petrolatum vehicle.[1] Furthermore, petrolatum is not an optimal vehicle for topical bioavailability of nickel, because the allergen is insoluble in petrolatum.

In the present investigation the cutaneous permeation of nickel from various hydrogels was compared with the standard patch test in petrolatum with a view to improving patch test materials.

Nickel chloride was used in the vehicles, as our previous work[2] has shown that the permeation rate of nickel from an aqueous solution is higher for nickel chloride than nickel sulphate. The permeation rate of nickel through excised human skin was examined and quantified by means of adsorption differential pulse voltammetry (ADPV) capable of detecting nickel in ultratrace amounts.

Correspondence: Dr Ann Fullerton.

To our knowledge quantitative data on the distribution of nickel in the various skin layers after topical application have not been reported previously. In the present study the nickel content in strips of stratum corneum, epidermis and dermis was determined.

METHODS

Chemicals

The following chemicals were used in the study: Nickel chloride hexahydrate, analytical grade; sodium chloride, suprapur grade quality (both from Merck, Darmstadt, Germany), methylcellulose 1500 (Dak 63, Denmark), hydroxypropyl methylcellulose (Methocel® E 4M, K 4M, F 4M) (Dow Chemical Co., Midland, MI, U.S.A.), polyoxyethylene-polyoxypropylene-copolymer (Pluronic® F-127) (Ugin Kuhlmann, France), polyvinyl alcohol (Polyviol® W 25/140) (Wacher-Chemie GmbH, Germany), and triethanolamine, 'zur synthese' quality (Merck-Schucchardt). The water used was purified by a Milli-Q deionization system (Millipore Corp., U.S.A.).

Test preparations

The following test preparations were used in the study: An aqueous solution of nickel chloride with a nickel content of 1·32 mg/ml, 5% nickel sulphate or 5% nickel chloride in petrolatum (Trolab, Hermal Chemie, Germany), and 5% nickel chloride in different aqueous hydrogels made using the following polymers: Gel A, methylcellulose (3%); gel B, Methocel® F 4M (4%); gel C, Methocel® K 4M (3%); gel D, Methocel® E 4M (3%); gel E, polyoxyethylene-polyoxypropylene-copolymer (30%); gel F, polyvinyl alcohol (10%); gel G, polyvinyl alcohol (10%) (with ethanol and triethanolamine).

Skin

Full thickness breast or abdominal skin was obtained from women undergoing surgery. The skin was stored at −20°C and allowed to thaw at room temperature before use. All subcutaneous fat was removed carefully and the skin surface was washed with saline.

Permeation procedure

Pieces of excised skin were mounted in glass diffusion cells with an available diffusion area of 1·8 cm^2. Details of the use of these diffusion cells have been presented previously.[2,3] The epidermal side of the skin was sealed with a glass stopper to make it airtight in order to simulate occlusion of the skin. The dermal side was bathed in saline as the recipient phase (7·5 ml).

Test solution (250 µl) or test ointment (approximately 28 mg; exact amounts were determined by differential weighing) was applied on the skin surface in the diffusion cells. The amount of nickel applied in the test preparations corresponded to the dose normally used in a Finn-Chamber, that is 9–16 mg 5% nickel sulphate in petrolatum, giving 139–228 µg nickel/cm^2.[4] All experiments were carried out at least in duplicate.

Recovery procedure

The ointment was removed from the skin surface by stripping the stratum corneum 5–10 times with adhesive tape (Micropore-3M® surgical tape). The epidermis was separated from the dermis by incubating the skin in a desiccator for 5 min at 60°C and 100% relative humidity, and the dermis was freeze dried. After wet ashing (see below) the nickel content in strippings, epidermis and dermis was determined quantitatively.

Analytical procedure

Nickel was quantified by adsorption differential pulse voltammetry (ADPV) according to the method of Pihlar *et al.*[5] This involves adsorption of the chelate nickel bis-dimethylglyoxymate onto a hanging mercury drop electrode in an ammonia buffer at pH 9·2. After a concentration step, a cathodic scan in the differential pulse mode reduces the chelate. The limit of detection is 1 ng/l. The instrumentation, conditions and reagents used were as previously described.[6] All analytical manipulations took place inside a laminar airflow clean bench to prevent airborne contamination.

Wet ashing

Prior to analysis the stripping tapes, epidermis and dermis sections were digested. The sections were placed in 30 ml Kjeldahl flasks, 0·2 ml H_2SO_4 (98%) and 1 ml HNO_3 (65%) (2 ml HNO_3 (65%) in the case of stripping tapes) were added and the flasks were heated on a sand bath at 170°C overnight. After addition of 1 ml H_2O_2 (30%) the flasks were heated to the point of thermal decomposition of sulphuric acid as seen by the white fumes expelled. After cooling, the digested samples were neutralized with NH_3 (25%) and made up to a volume of 5 ml. To 0·5 ml aliquots of a dilution of this solution were added 8·5 ml milli-Q water, 1·0 ml ammonia buffer (1M) and 10 μl dimethylglyoxim (0·1M). The nickel content was quantitated by means of two standard additions. A blank value of 2·23 ± 0·39 ng ($n = 7$) has been subtracted from the results.

RESULTS

Effect of vehicles

Figures 1a and 1b show the influence of the various vehicles by following the build-up of nickel in the recipient phase as a function of time. The permeation of nickel was strongly influenced by the vehicle. With petrolatum it was not possible to detect nickel in the recipient phase if the nickel was in the form of the sulphate. The higher cutaneous bioavailability of nickel chloride compared with nickel sulphate is in accordance with our earlier observations of permeation of nickel from aqueous solutions.[2]

The gels made from methylcellulose and its hydroxypropyl derivates (gels A–D) showed increased nickel permeation rates with significantly higher cutaneous bioavailability of nickel than was found with the petrolatum vehicle.

The nickel permeation from gel D was almost as fast as from the aqueous solution. However, in patch testing aqueous solutions are applied on small filter paper discs in a Finn Chamber, thus a hydrogel would be a more convenient formulation and at the same time has the advantage of being an aqueous solution. Furthermore, gel D is the only transparent methylcellulose gel and makes visual inspection possible. Consequently, the subsequent studies were carried out using gel D as the vehicle.

When using nickel chloride in polyoxyethylene-polyoxypropylene-copolymer gel (gel E) no permeation was observed. This is probably because the nickel ion is kept in micelles in the gel due to the high polymer concentration (30%); thus the liberation rate from the gel may be reduced. A deaccelerating effect was also seen when triethanolamine was added to the polyvinyl alcohol gel (gel G) making it alkaline (pH = 10·2).

Effect of occlusion

In Figure 2 the effect of occlusion on the permeation rate from gel D can be seen. The skin was occluded for 48 h as in a normal patch test procedure. At 48 h the occlusion was removed from

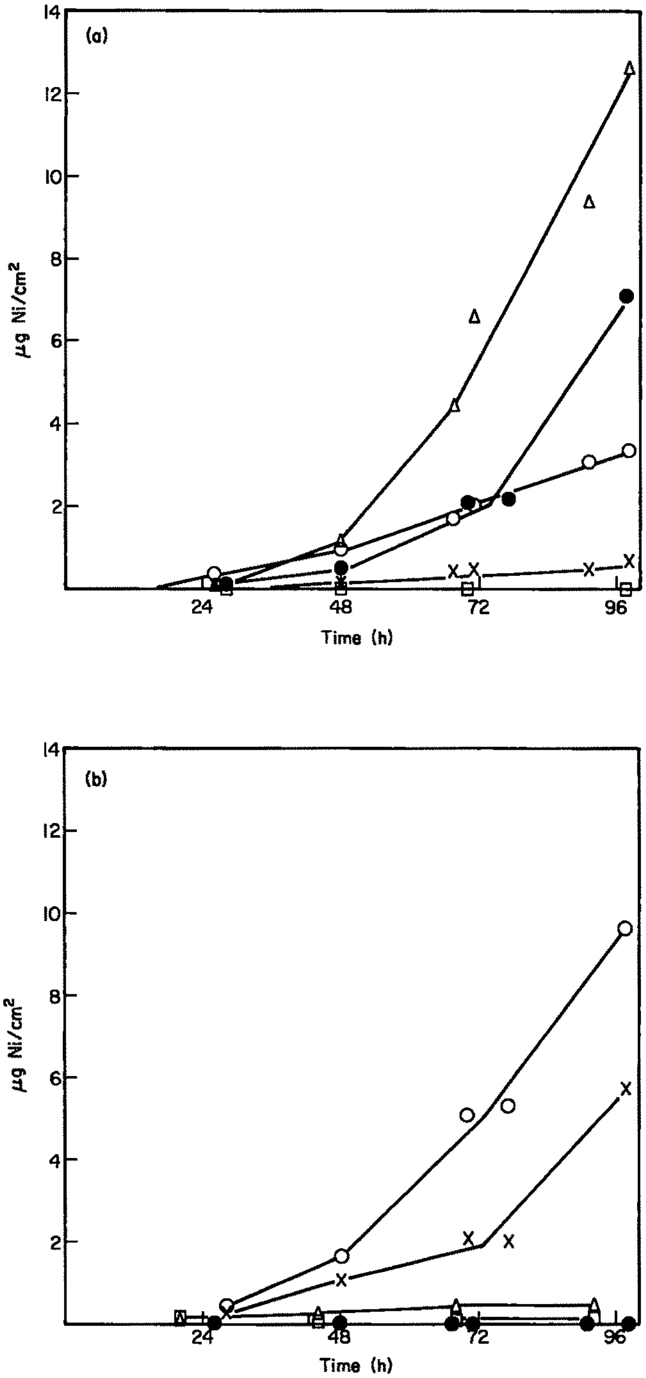

FIGURE 1. Effect of vehicles on the permeation rate of nickel through human skin *in vitro*. (a) △, aqueous $NiCl_2$; □, $NiSO_4$ in petrolatum; ; ×, $NiCl_2$ in petrolatum; ●, $NiCl_2$ in gel A; ○, $NiCl_2$ in gel B. (b) ×, $NiCl_2$ in gel C; ○, $NiCl_2$ in gel D; ●, $NiCl_2$ in gel E; △, $NiCl_2$ in gel F; □, $NiCl_2$ in gel G.

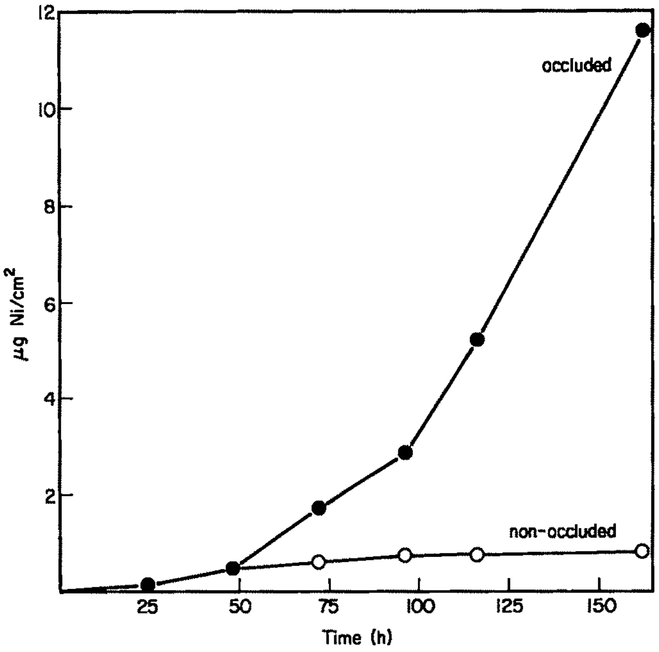

FIGURE 2. The effect of occlusion on the permeation of nickel from gel D *in vitro*. ●, occlusion maintained; ○, occlusion removed at 48 h.

half of the samples and excess nickel salt on the skin surfaces was carefully removed by gentle wiping with medical tissue. The permeation profiles emphasize the importance of occlusion during the first 48 h of a patch test to ensure cutaneous bioavailability.

Nickel content in skin layers

The content of nickel in various layers of human skin after percutaneous application of 5% nickel chloride in gel D for 96 h under occlusion was determined. Table 1 shows the nickel content in strippings, epidermis, dermis and the receipient phase.

As a consequence of the barrier function of the skin a large amount of the applied nickel remained on the skin surface and in the upper part of the stratum corneum. The amount of nickel found in the strippings gradually declined through the stratum corneum. A significant amount of nickel was found in the dermis, 5·2 μg/1·8 cm² corresponding to 1·6% of the applied dose. Although the dermis represents a larger distribution volume compared with the thin epidermal layer, in a ratio of 10:1, and has a high content of water, nickel seemed to accumulate in the epidermis (35·8 μg/1·8 cm²). This is probably due to epidermal binding of nickel and is in accordance with the observations of Samitz and Katz,[8] who found considerable amounts of nickel bound to powdered epidermis.

Effect of nickel concentration

The penetration of nickel was also studied with different percentages of nickel in gel D. The results are illustrated in Figures 3a and 3b.

Figure 3a shows the amount of nickel in the recipient phase at 48 h and 96 h as a function of

TABLE 1. The recovery of nickel from various layers of human skin and from the recipient phase after application of 5% nickel chloride in gel D for 96 h under occlusion

Layer		μg Ni/1·8 cm²	% of applied dose
Stripping*	1	125·2	37·3
"	2	17·8	5·3
"	3	5·8	1·7
"	4	6·3	1·9
"	5	4·4	1·3
"	6	3·1	0·9
"	7	2·9	0·9
"	8	2·9	0·9
"	9	2·5	0·7
"	10	1·8	0·5
Total in strippings		172·7	50·9
Epidermis		35·8	10·6
Dermis		5·2	1·6
Recipient phase		1·2	0·4
Total Ni recovered		214·9	63·8

* The skin was not washed before stripping.

nickel concentration in gel D. It can be seen that the application of higher concentrations resulted in higher penetration rates. In order to evaluate the amount of nickel remaining in the skin layers as a function of nickel concentration, human skin exposed for 96 h was stripped five times and separated into epidermis and dermis. As shown in Figure 3b the amounts of nickel in the 4th and 5th strippings, epidermis and dermis were linearly related to the concentrations of nickel chloride in the gel. Therefore, the nickel concentration used in a patch test preparation of gel D is an important consideration. The distribution of nickel in this experiment was in accordance with the results presented in Table 1 showing significant binding of nickel in the epidermis.

COMMENT

Nickel chloride (5%) or nickel sulphate (5%) in petrolatum was compared with various hydrogels and an aqueous solution of nickel chloride. In order to achieve good conditions for penetration the vehicle should adhere tightly to the skin surface and nickel should be almost, or better still, completely dissolved in the vehicle, as shown by Wahlberg,[7] who found a higher penetration rate of a metal ion from a solution than from a semisolid suspension. Furthermore, the backing should be occlusive to increase the penetration of nickel, as we found in our earlier study that occlusion gives rise to a significantly higher permeation rate compared with the non-occluded situation.[2]

The hydrogels are solvent-polymer systems with water used as the solvent. The polymers chosen were non-ionic to avoid influencing the activity or stability of the preparation. They were all presumed to be non-allergenic and non-irritant and to have good film forming properties. The various hydrogels leave the nickel spread across the skin surface in a thin film without

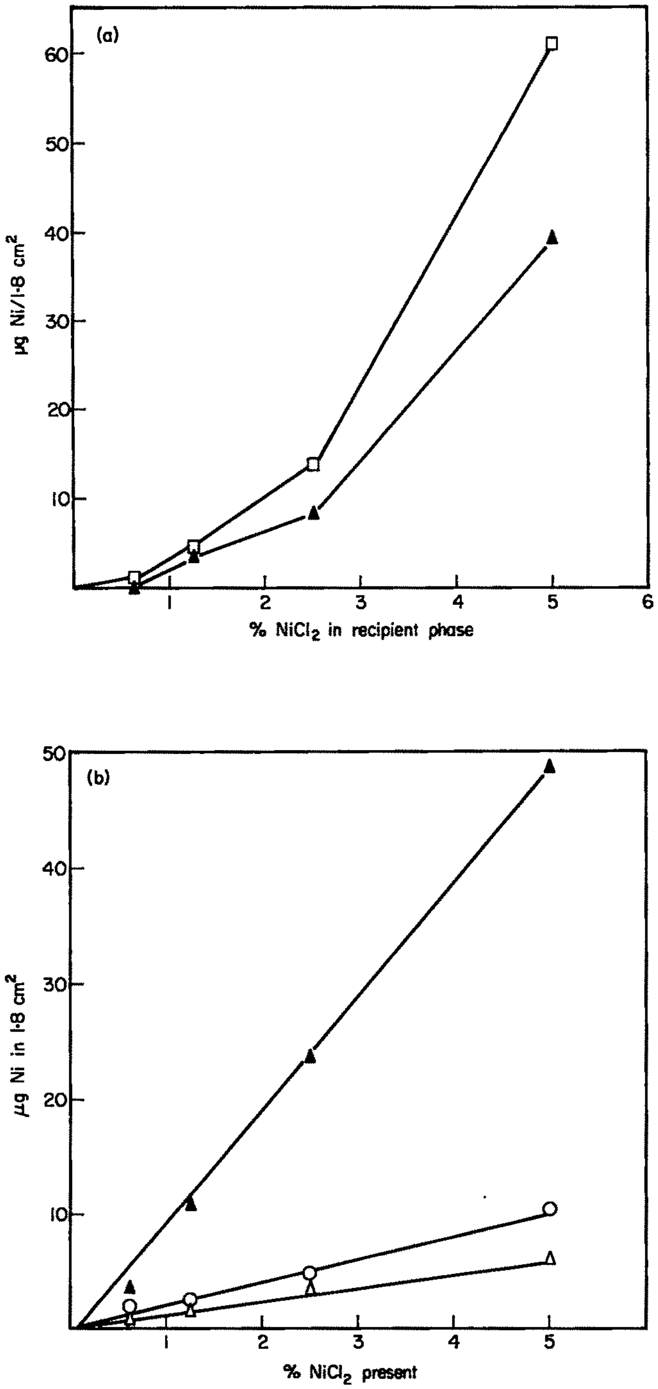

FIGURE 3. (a) The amount of nickel in the recipient phase as a function of the nickel chloride concentration in gel D. ▲, at 48 h; □, at 96 h. (b) The amount of nickel in the 4th and 5th stripping (○), epidermis (▲) and dermis (△) at 96 h as a function of the nickel chloride concentration in gel D.

microscopically detectable crystals. A microscopic examination of nickel chloride or nickel sulphate in the petrolatum vehicle showed unevenly dispersed irregular crystals in the range 25–100 μm. The viscosity of the hydrogels makes them superior to aqueous solutions as patch test vehicles because they are easily applied and retained on the skin surface.

The use of hydrogels makes it possible to keep nickel in solution and at the same time achieve a consistency which is suitable for patch testing. From our study it is obvious that the vehicle is extremely important to the cutaneous availability of nickel. The vehicle has to be considered carefully with respect to solubility, stability and compatibility with the allergen. For example, nickel causes the Carbopol® polymer gels to precipitate, thus making it impossible to obtain a proper gel. On the basis of the present *in vitro* study the Methocel® E 4M gel (gel D) appears the most promising choice for nickel, and it is currently being investigated *in vivo* using the Finn Chamber patch test technique. The results so far appear to indicate that the nickel concentration can be reduced at least fivefold compared with the concentration required in a petrolatum vehicle. The gel may also be suitable for other hydrophilic allergens, for example cobalt and formaldehyde.

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Influence of age and severity of dermatitis on the percutaneous absorption of hydrocortisone in children

M.TURPEINEN

Department of Allergic Diseases, University Central Hospital, Helsinki, Finland

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SUMMARY

The results of 55 4-h hydrocortisone absorption tests in 38 children with atopic or seborrhoeic dermatitis were analysed to evaluate the effect of age and severity of the dermatitis on percutaneous absorption of hydrocortisone. The children were divided into three groups on the basis of the severity of the dermatitis. The absorption of hydrocortisone caused a significantly higher mean rise of serum cortisol in 20 children with severe dermatitis (Group A) than in 17 children with moderate dermatitis (Group B). The mean post-application rise of serum cortisol in 18 children with mild dermatitis (Group C) was significantly lower than in the children with moderate dermatitis. There was a significant negative linear correlation between age and the post-application rise of serum cortisol in Groups A and C. In these groups the mean post-application rise of serum cortisol was significantly higher in children aged under 18 months than in children aged 18 months or over. Severe widespread dermatitis and an age under 18 months are two relevant risk factors in the topical use of hydrocortisone.

Most studies of the barrier function of normal skin have not shown any significant difference in percutaneous absorption between adults and children, except pre-term infants.[1–4] However, increased sensitivity to systemic side effects has been reported in infants and children with diseased skin treated with potent topical glucocorticoids.[5–10] Systemic side effects of topical hydrocortisone have been reported in children,[5,11–14] but not to our knowledge in adults. This discrepancy between children and adults has been explained by the greater systemic availability of the drug due to a greater surface area to weight ratio in children.[15] The purpose of the present study was to evaluate the influence of the age of the child and the severity of the dermatitis on percutaneous absorption of hydrocortisone, using a new direct method.[14]

Correspondence: Dr M. Turpeinen, Department of Allergic Diseases, University Central Hospital, Meilahdentie 2, 00250 Helsinki, Finland.

METHODS

Patients

The percutaneous absorption of hydrocortisone was studied in 38 children with atopic or seborrhoeic dermatitis of varying severity. In 17 children the test was performed twice during different phases of the dermatitis. Hence, the total number of absorption tests was 55. The extent of skin involvement was determined using a burn assessment chart.[16] The condition of the skin was assessed by scoring erythema and excoriations on a scale from 0–3; 0 = none, 1 = mild, 2 = moderate and 3 = severe.

On the basis of the severity of the dermatitis the chilren were divided into the following three groups: Group A: 20 children with widespread high grade dermatitis (⩾40% skin surface involvement and sum of scores for erythema and excoriations 4–6), Group B: 17 children with widespread low grade dermatitis (sum of scores for erythema and excoriations 0–3). Group C: 18 children with low grade dermatitis covering less than 40% of the body surface.

To evaluate the influence of age on percutaneous absorption the children were further divided into two subgroups: age under 18 months and 18 months or over. In Group A there were 10 children, four girls and six boys, in each subgroup. The mean age was 8½ months (range 1–15 months) in the younger group and 7·2 years (range 3·5–11·9 years) in the older group. In Group B there were eight children (one girl and seven boys) aged under 18 months and nine children (six girls and three boys) aged 18 months or over. The mean ages were 5 months (range 2–8 months) and 6·9 years (range 2·2–14·4 years), respectively. In Group C there were 10 children (five girls and five boys) in the younger group and eight children (three girls and five boys) in the older group. The mean ages were 7 months (range 1–15 months) and 6·2 years (range 4·1–11·2 years), respectively. Clinical details of these groups of patients are presented in Table 1.

The absorption test

The percutaneous absorption of hydrocortisone was measured under dexamethasone suppression using the 4 h absorption test.[14] The basal serum cortisol prior to the application of hydrocortisone cream varied from 18 to 204 nmol/l (mean ± SD 66 ± 40 nmol/l). The criterion for adrenocortical suppression was a basal serum cortisol level of less than 200 nmol/l. This was

TABLE 1. Clinical details of the three groups of children studied. Values are means ± SD

		No of patients					
Age		Seborrhoeic dermatitis, atopic dermatitis	Weight (kg)	Skin surface area (m^2)	% skin surface covered by dermatitis	Sum of the dermatitis scores	Amount of hydrocortisone cream used in the test (g)
Group A	< 18 months	6,4	7·3 ± 2·1	0·35 ± 0·07	79 ± 16	4·3 ± 0·5	15·6 ± 4·7
	⩾ 18 months	10,0	27·3 ± 12·9	0·96 ± 0·30	83 ± 6	4·5 ± 0·7	45·6 ± 17·2
Group B	< 18 months	5,3	6·8 ± 1·2	0·33 ± 0·05	69 ± 13	2·1 ± 0·8	12·9 ± 3·2
	⩾ 18 months	9,0	27·4 ± 17·1	0·94 ± 0·41	70 ± 19	2·1 ± 0·9	53·7 ± 26·6
Group C	< 18 months	6,4	7·1 ± 2·0	0·35 ± 0·07	13 ± 10	1·2 ± 0·7	14·6 ± 5·9
	⩾ 18 months	8,0	23·7 ± 11·5	0·86 ± 0·24	9 ± 8	0·9 ± 0·6	49·3 ± 23·9

achieved in all, but one child. The percutaneous absorption of hydrocortisone was expressed as the maximum post-application rise in serum cortisol.

Statistical analysis

The relationship between hydrocortisone absorption and age was evaluated using a regression analysis. Mean values in the three groups were compared using Student's *t*-test for unpaired observations. The individual absorption results were adjusted for the ratio of skin surface area to weight by dividing the maximum post-application rise in serum cortisol by this ratio.

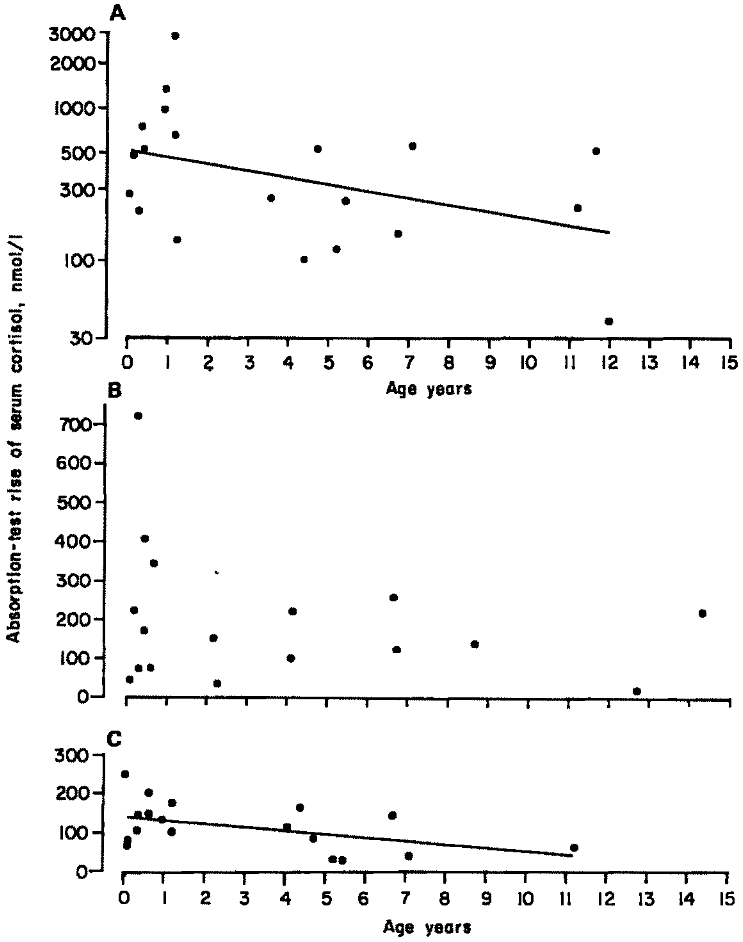

FIGURE 1. The correlation between the age of the child and the post-application rise in serum cortisol. A = widespread, high grade dermatitis ($n = 20$), B = widespread, low grade dermatitis ($n = 17$), C = low grade dermatitis covering less than 40% of the body surface ($n = 18$). There was a significant negative correlation in A ($r = -0{\cdot}483$; $P < 0{\cdot}05$) and C ($r = -0{\cdot}469$; $P < 0{\cdot}05$). Note log scale in A.

TABLE 2. The post-application rise of serum cortisol (nmol/l) in children with severe (A), moderate (B) and mild (C) dermatitis. Values are means ± SD

	Children Aged		
	< 18 months	≥ 18 months	All children
A	807 ± 718* (*n* = 10)	268 ± 204 (*n* = 10)	537 ± 589† (*n* = 20)
B	257 ± 218 (*n* = 8)	135 ± 81 (*n* = 9)	193 ± 175‡ (*n* = 17)
C	132 ± 56** (*n* = 10)	78 ± 54 (*n* = 8)	107 ± 61 (*n* = 18)

Significantly greater than in children aged 18 months or over $*P<0·025$, $**P<0·05$

† Significantly greater than in Group B, $P<0·025$

‡ Significantly greater than in Group C, $P<0·025$

TABLE 3. The post-application rise of serum cortisol adjusted for individual skin surface area to weight ratios (nmol/l/m^2/kg) in children with severe (A), moderate (B) and mild (C) dermatitis. Values are means ± SD

	Children Aged		
	< 18 months	≥ 18 months	All children
A	16798 ± 15599* (*n* = 10)	7211 ± 4901 (*n* = 10)	12004 ± 12516** (*n* = 20)
B	5328 ± 4559 (*n* = 8)	3682 ± 2450 (*n* = 9)	4456 ± 3780† (*n* = 17)
C	2665 ± 1122 (*n* = 10)	2018 ± 1292 (*n* = 8)	2378 ± 1240 (*n* = 18)

* Significantly greater than in children aged 18 months or over, $P<0·05$.

** Significantly greater than Group B, $P<0·025$.

† Significantly greater than Group C, $P<0·05$.

RESULTS

The effect of severity of dermatitis on the adsorption of hydrocortisone

The mean post-application rise in serum cortisol was significantly higher in Group A (severe dermatitis) than in Group B (moderate dermatitis) ($P < 0{\cdot}025$), and also significantly higher in Group B than in Group C (mild dermatitis) ($P < 0{\cdot}025$). The results are presented in Table 2.

The effect of age on the absorption of hydrocortisone

The individual maximum post-application rises in serum cortisol in patients in the three groups are shown in Figure 1. In Groups A and C a significant negative linear correlation was found between the rise in serum cortisol and age ($r = -0{\cdot}483$; $P < 0{\cdot}05$ and $r = -0{\cdot}469$; $P < 0{\cdot}05$, respectively). In Group B no significant correlation was found ($r = -0{\cdot}251$; $P > 0{\cdot}05$). In Groups A and C the mean post-application rise in serum cortisol was significantly higher in children aged under 18 months than in children aged 18 months or more ($P < 0{\cdot}025$ and $P < 0{\cdot}05$, respectively). In Group B the rise in serum cortisol was higher in children aged under 18 months than in children aged 18 months or over, but the difference was not statistically significant ($P < 0{\cdot}10$). The results are presented in Table 2. The individual absorption values were also adjusted for the ratio of skin surface to weight by dividing the rise in serum cortisol by this ratio. After this adjustment the difference in the mean absorption between the two age groups was still significant in Group A ($P < 0{\cdot}05$) but not in Group C. The adjusted means are presented in Table 3.

DISCUSSION

The results of the present study show that the percutaneous absorption of hydrocortisone increased significantly with increasing severity of the dermatitis. This has also been suggested previously by Feinblatt *et al.*[12] Using monkey skin *in vitro* Bronaugh *et al.*[17] found that percutaneous absorption of hydrocortisone through eczematous skin was twice as high as through normal skin. Our finding of a twofold difference of absorption between patients with mild and moderate dermatitis is in accordance with this. Furthermore, the results of the present study show that the difference in absorption between mild and severely inflamed dermatitis can be as high as fivefold.

In the present study the percutaneous absorption of hydrocortisone was found to decrease significantly with increasing age of the child, both in mild and in severe dermatitis. The post-application rise in serum cortisol was signficantly higher in children aged under 18 months than in children aged 18 months or over. Wester *et al.*[15] explained the age dependent difference in terms of the different skin surface area to weight ratio. This could explain the disappearance of the difference in the absorption between the two age groups in the case of mild dermatitis, when the absorption was adjusted for this factor. In severe dermatitis, however, the adjusted absorption was still significantly higher in younger children compared with older children. It is possible that the severe inflammation of the skin impairs the skin barrier to hydrocortisone more profoundly in infants than in older children.

On the basis of the present results it can be concluded that severe dermatitis is associated with high percutaneous absorption of hydrocortisone. The absorption is further increased in young infants. Thus, a special group with high percutaneous absorption can be identified, that is children aged under 18 months with severe widespread dermatitis. In these children the post-application rise of serum cortisol equalled the serum levels of cortisol obtained with maximal stimulation of normal adrenals in the ACTH test.[18] High percutaneous absorption of hydrocortisone has in turn been found to be associated with adrenocortical suppression.[14] The development of systemic side-effects can be expected if the dermatitis is not brought into remission, and the absorption continues at a high level. On the other hand in the case of mild dermatitis, also in infants, the absorption of hydrocortisone and also the risk of systemic side effects seems to be low.

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The effect of the vehicle formulation on the stratum corneum penetration characteristics of clobetasol 17-propionate *in vivo*

W.S. WATSON AND A.Y. FINLAY*

Department of Clinical Physics and Bioengineering and *Department of Dermatology, Southern General Hospital, Glasgow, U.K.

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SUMMARY

The multiple skin surface biopsy technique to measure stratum corneum (SC) penetration characteristics *in vivo* has been more accurately quantified and used to provide information on the kinetics of transfer of a topical steroid through the SC. Radiolabelled clobetasol 17-propionate formulated in two different vehicles, consisting primarily of dibutyl adipate or of propylene glycol, was applied and the penetration characteristics compared. Using exponential expressions for radioactivity in skin surface biopsies as a function of SC depth, and total SC radioactivity as a function of time, a mathematical model was developed to calculate the rate of loss of steroid through superficial shedding and the rate of transfer of steroid from the SC into the epidermis. When a topical steroid was applied for 8 h under gauze occlusion, over 80% was recovered from the gauze.

Finlay and Marks[1] have described a technique to measure stratum corneum (SC) penetration characteristics using multiple skin surface biopsies. We have improved the accuracy of this procedure by exactly measuring the amount of radiolabelled steroid initially applied, and by quantifying the amount of steroid lost from the surface. It is, therefore, now possible to relate intracorneal concentration of steroid to initial application values, removing a major source of error. We describe here a method for calculating rates of transfer of steroid from the SC and propose a method for correcting the results to take into account SC turnover. The improved technique and analysis methods have been used to compare the SC penetration characteristics of the same radiolabelled steroid, clobetasol 17-propionate, in two different vehicles.

Correspondence: Dr A.Y.Finlay, Department of Medicine (Dermatology), University of Wales College of Medicine, Heath Park, Cardiff CF4 4XN, U.K.

METHODS

Ten male volunteers aged over 40 years gave their written informed consent to take part in the study, which was approved by the local Ethical Committee and the D.H.S.S. Administration of Radioactive Substances Advisory Committee. Each subject had six squares (sites 1–6) measuring 2 × 2 cm, and 4 cm apart, drawn with a ballpoint pen on the upper back. A measured weight of ointment (approximately 25 mg) was applied to each square. Five subjects had tritium radiolabelled (16, 16a-^{3}H) clobetasol 17-propionate (CP) 0·05% (Amersham International plc, Little Chalfont, Bucks, U.K.) applied in a commercial ointment formulation (PG) consisting of CP dissolved in propylene glycol and dispersed in a paraffin. The other five subjects had a formulation applied consisting of tritium radiolabelled CP 0·05% dissolved in a low viscosity oil di-n-butyl adipate, wax and liquid paraffin matrix (BA).

The PG and BA ointment preparations were supplied in 3 g tubes with a narrow nozzle. For each application the ointment was evenly squeezed out to an approximate length of 2 cm onto the outside base of a round bottomed polypropylene sample tube which was then weighed. This sample tube was used to directly apply and evenly spread the ointment across the square on the subject's back and the tube was then reweighed. On each occasion that a group of subjects entered the study, similar control samples of ointment were weighed and their radioactivity later counted at the same time as the subject samples. The ^{3}H activity applied to each site was approximately 0·27 MBq.

After application of the ointment, each site was covered with a dry folded gauze dressing (Swabs BP Gauze 12, 10 × 10 cm, 12 ply) which was secured in position with adhesive tape. Eight hours after the application the dressings were removed; their radioactivity was later counted. At this same time, five consecutive skin surface biopsies (SSBs) (A, B, C, D and E) were taken from site 1 using glass slides coated with cyanoacrylate glue (Powabond, Double H International, Staines, Middx, U.K.). Further series of five SSBs were taken from sites 2, 3, 4, 5 and 6 at 12, 24, 48, 96 and 168 h respectively. It was assumed that the SC at each site was completely removed in five SSBs of equal thickness.

For each SSB the biopsy was removed from the slide using ethyl acetate and the radioactivity counted. Aliquots of solvent extractions of the swabs and of dissolved control sample solutions were also assayed for tritium activity. These estimations were carried out by Glaxo Group Research Ltd., Greenford, Middx, U.K.

The tritium radioactivity from each SSB and each swab was expressed as a percentage of the radioactivity applied at the corresponding site (% applied dose). This latter radioactivity could be calculated from the weight of ointment applied to the site and the radioactivity per g of the control sample.

Statistical analysis

The data were analysed using the Wilcoxon rank sum test.

RESULTS

In presenting the results, the assumption was made that the tritium tracer is not removed from the CP molecule to any significant extent, so that tritium radioactivity can be directly related to CP concentration. Figures 1a and 1b show the mean CP concentrations in the SSBs for the PG and BA formulations as a function of SC depth at the various biopsy times. It can be seen that the steroid concentration decreased with increasing depth into the SC and also with time. The

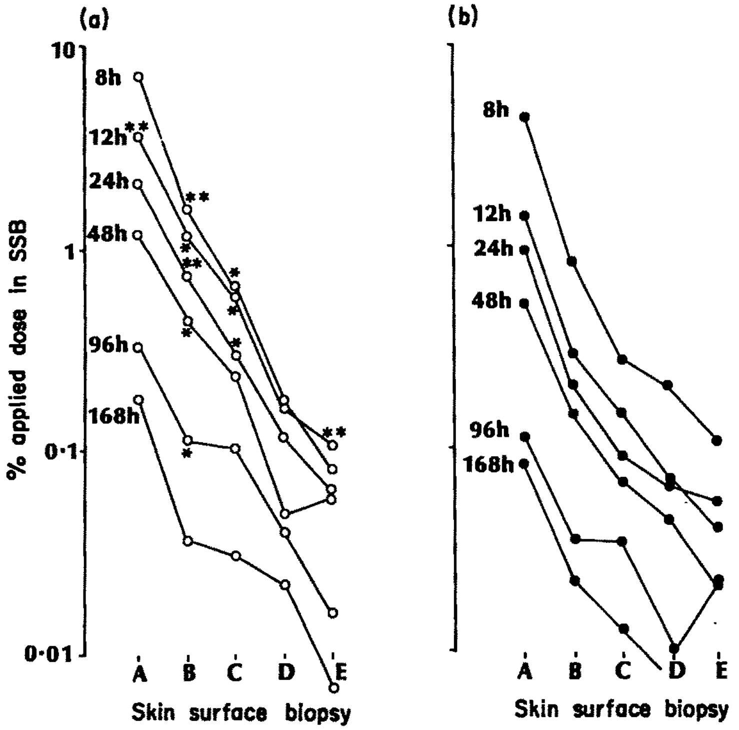

FIGURE 1. Concentration of radiolabelled steroid shown as a function of SC depth and time for (a) the PG formulation and (b) the BA formulation. Each point represents the mean of five estimations. Total amount applied to skin at time 0 = 100%. A is the outermost biopsy and E the innermost. Each SSB is considered to be one 'depth unit' thick.
Significantly different from BA $^{*}P<0{\cdot}01$, $^{**}P<0{\cdot}05$.

decrease with SC depth at a given biopsy time was essentially monoexponential, i.e. the variation of activity with depth can be expressed mathematically as follows:

$$A(x)=A(0)\exp(-\mu x)$$

where $A(x)$ is the radioactivity at SC depth x, $A(0)$ is the radioactivity at the surface (i.e. $x=0$) and μ is a constant in reciprocal 'depth units'.

This means that by taking the logarithm of the CP concentration and plotting this against the SC depth, almost linear plots are obtained as shown in Figure 1. When linear regression analyses of log CP concentration against SC depth were performed, statistically significant linear correlations were obtained for all concentration profiles ($P<0{\cdot}03$) with the exception of the profile for the BA formulation at 96h ($P=0{\cdot}09$). The profiles for the two formulations were generally similar; however the PG formulation yielded significantly higher CP concentrations than the BA formulation at several SC levels and biopsy times (Fig. 1a). When interpreting Figure 1 it should be noted that the high levels in the outermost SSB (A) include radioactivity of drug persisting on the surface of the SC as well as drug which has permeated into this first layer.

The total tritium radioactivity in the SC at a given biopsy time was obtained by adding the activities of the five SSBs A, B, C, D and E taken at that time. Figure 2 shows the variation of the mean total SC activities, and therefore CP concentrations, with time for the two formulations.

Generally, the PG formulation yielded higher activities than the BA formulation, the difference between the formulations being significant at 8, 12 and 96 h ($P=0{\cdot}05$, $P=0{\cdot}01$ and $P=0{\cdot}05$, respectively). The variation of total SC radioactivity with time can be expressed mathematically as the sum of two exponential functions i.e.

$$\text{Total SC activity} = \text{A} \exp(-\alpha \text{T}) + \text{B} \exp(-\beta t)$$

Where A and B are in units of % applied dose, α and β are in units of reciprocal hours and t is time in hours. By applying a mathematical curve fitting routine to the data for each formulation, the following expressions are obtained:

PG formulation: $\text{Total SC radioactivity} = 27{\cdot}1 \exp(-0{\cdot}20\, t) + 5{\cdot}25 \exp(-0{\cdot}021\, t)$

BA formulation: $\text{Total SC radioactivity} = 2294{\cdot}6 \exp(-0{\cdot}80\, t) + 2{\cdot}34 \exp(-0{\cdot}021\, t)$

These fitted curves are also shown in Figure 2. It should be noted that although these curves provide good fits to the data between 8 and 168 h, extrapolating them to shorter or longer times would not be appropriate.

The rate of loss of steroid from the SC was calculated approximately for each time period for each subject as follows: Rate of loss of steroid from SC = (Am − An)/(tm − tn)% dose/h where

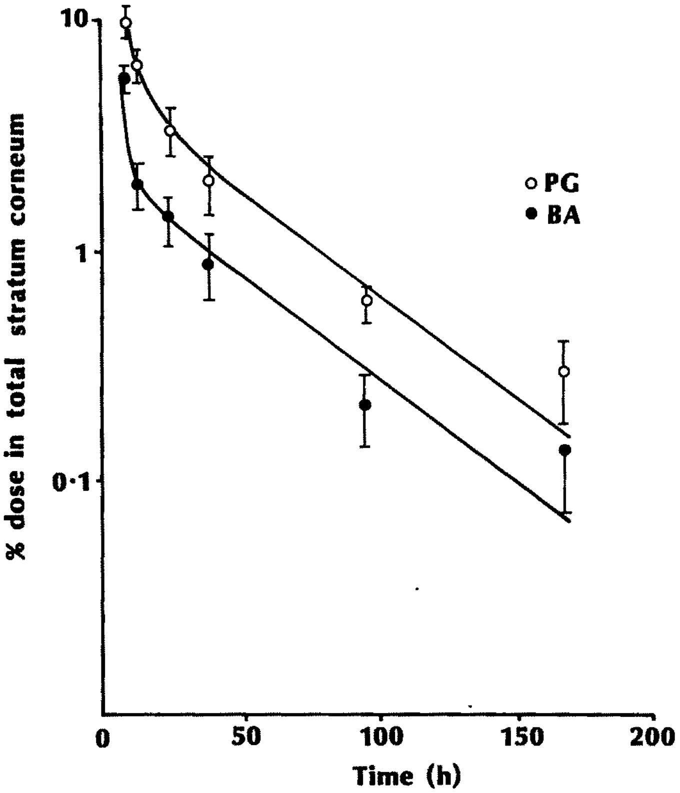

FIGURE 2. Total steroid concentration in the stratum corneum as a function of time. Each point represents the mean of five sets of measurements ± SEM. The curves are double exponential fitted lines. The two formulations are significantly different at 8 h ($P<0{\cdot}05$), 12 h ($P<0{\cdot}01$) and 96 h ($P<0{\cdot}05$).

Am and An were the total SC radioactivities at the two 'adjacent' biopsy times tm and tn, e.g. 8 and 12 h or 96 and 168 h. For both formulations the rate of steroid loss fell rapidly with time with inter-formulation differences only being significant for the 12–24 h time period, where the loss rate was greater for the PG formulation ($P < 0{\cdot}01$).

The rate of loss of steroid can also be expressed as a fraction of the average steroid radioactivity for each time interval i.e. fractional rate of steroid loss from SC = 100 (Rate of steroid loss from tm to tn)/0·5 (Am + An)%h.

The reason for expressing the data in this way is that it eliminates the effect of differing amounts of ointment remaining on the occluding swab for each formulation and, therefore, gives more information about the steroid kinetics in the SC.

The level of radioactivity on the gauze swabs removed at 8 h, expressed as a percentage of the total radioactivity applied at the corresponding site, indicated that a large proportion of the steroid was to be found in the swabs at this time. A mean (± SD) of 80·8 ± 12·2% ($n = 30$) of the PG formulation was recovered and 91·1 ± 9·7% ($n = 30$) of the BA formulation.

ANALYSIS

In an attempt to express the above results in a more cohesive way and also to provide a general technique for the assessment of steroid kinetics in the SC, a mathematical analysis was applied to the results which allows the inward diffusion and outward desquamation loss rates to be calculated as a function of time. The data in the present study was too limited in terms of time period covered and patient numbers for the analysis to be performed in a rigorous statistical sense and, therefore, the differences seen must be considered in this instance as qualitative rather than quantitative. Therefore, the present analysis may be considered as a preliminary demonstration of the potential value of this new analytical method.

As stated above, the variation of total steroid radioactivity in the SC as a function of time can be expressed, for each steroid formulation, as the sum of two exponential functions of time. Using calculus, these exponential expressions can be differentiated with respect to time to yield expressions for the rate of change of steroid activity with time i.e. the rate of loss of steroid activity from the SC with time. If it is assumed that the steroid leaves the SC either inwards along the diffusion gradient or by desquamation outwards then: Total steroid loss rate = loss rate due to inward diffusion + loss rate due to outward desquamation.

Since the total loss rate has been obtained by differentiation, only one of the loss rates on the right hand side of the above equation need be calculated. The loss rate due to desquamation was the easier to calculate. If the SC is completely replaced in Y days, then in 1 h the thickness of the SC desquamated is $5/(24 \times Y)$ (the SC being 5 'depth units' thick). An average value[2] for the SC turnover time on the upper back is 23·6 days which means that 0·0088 'depth units' are lost per hour from the outermost surface of the skin. Returning to Figure 1, this is equivalent to moving the radioactivity profiles to the left relative to the axes by 0·0088 'depth units' per hour and so losing from the skin surface the activity which 'crosses' the Y-axis in that time. This radioactivity can be estimated for each biopsy time by again using calculus to integrate the monoexponential functions of SSB activity versus SC depth from $x = 0$ to $x = 0{\cdot}0088$ 'depth units' thus yielding the SSB activity lost per hour due to desquamation, i.e. the loss rate due to desquamation. The diffusion loss rate (in units of % dose applied /h) was then obtained by subtraction using the above formula. The results are shown in Figure 3, where it can be seen that the BA formulation initially, i.e. at 8 h, had a higher inward diffusion loss rate but that at all other times the loss rate was less than for the PG formulation. The desquamation loss rates at 8 h

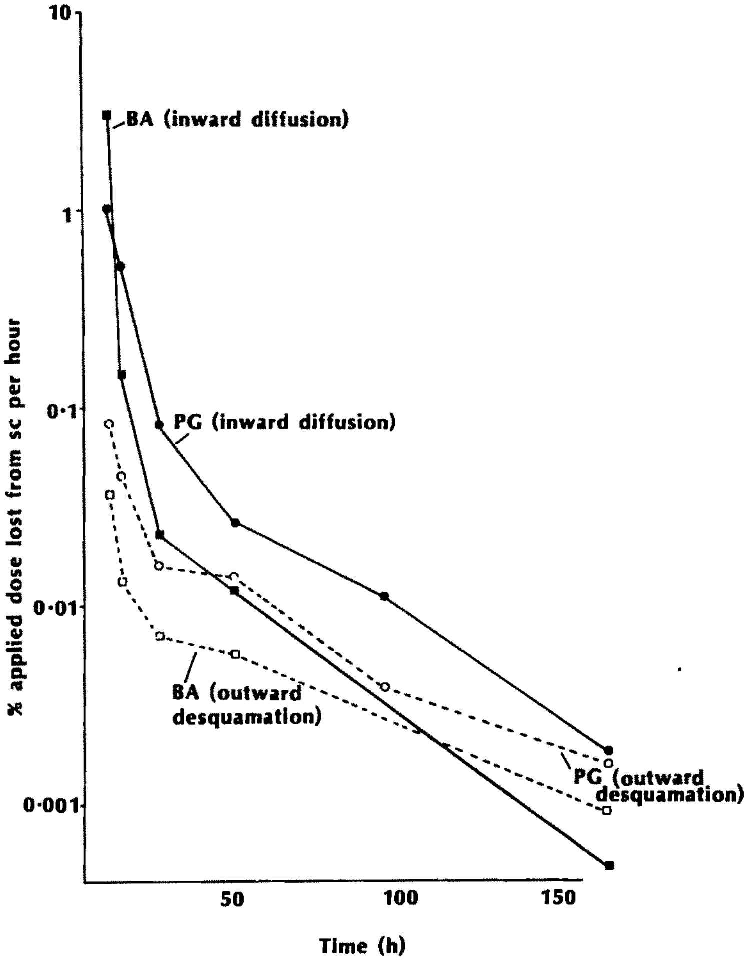

FIGURE 3. Rate of loss of steroid in the two formulations from the stratum corneum by diffusion into the underlying epidermis, and by continuous loss of stratum corneum from the surface.

represent only between 1 and 8% of the total loss rate. However, by 48h approximately 30% of the loss rate was by desquamation.

The loss rates can also be calculated as fractional loss rates, as described above, by dividing the calculated loss rate at any time by the calculated SC activity at that time. As indicated above, this yields information about the behaviour of the steroid in the SC unaffected by the amount of ointment taken up by the occluding swab. The results are illustrated in Figure 4 where it can be seen that at 8 h the BA formulation is being lost from the SC at a much higher rate than the PG formulation. However, at all later times there is little difference between the formulations. In terms of desquamation loss rates, it can be seen that approximately 0·7% of the SC activity is lost per hour for each formulation and that this percentage is relatively constant up to 96 h with perhaps a rise at 168 h.

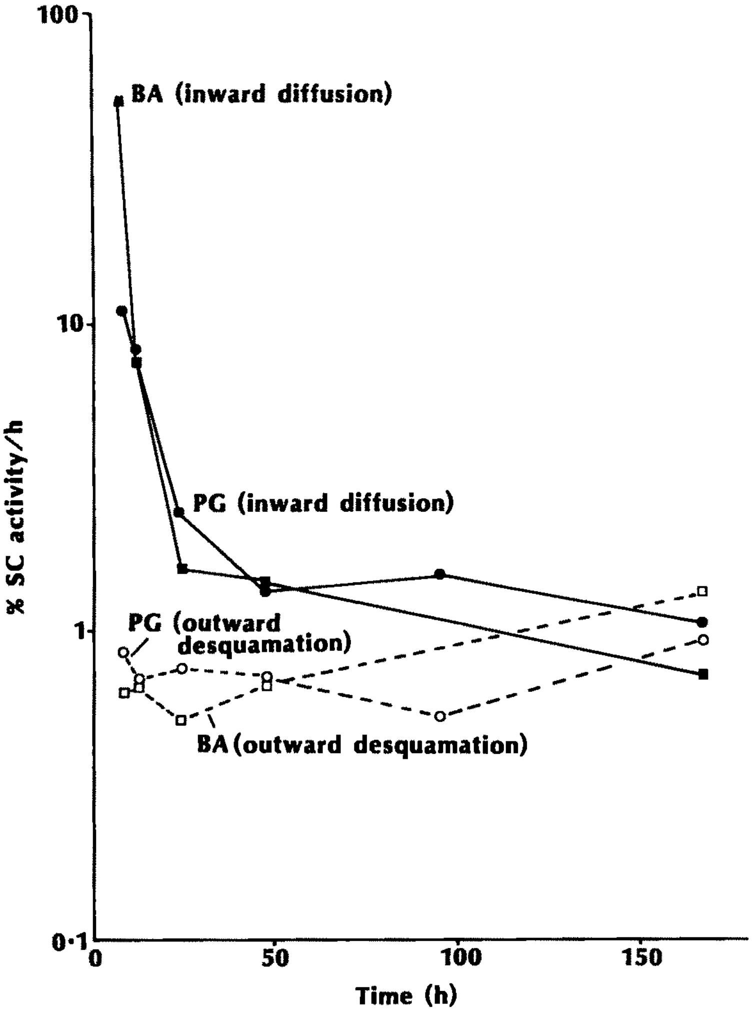

FIGURE 4. Rates of loss of steroid from the stratum corneum in relation to the concentration of steroid in the stratum corneum.

DISCUSSION

This study has demonstrated how the SSB technique can be used to give information about the rate of transfer of steroid from the SC to the rest of the underlying skin.

It has been thought that dibutyl adipate would release steroid more slowly than propylene glycol because of a higher 'vehicle/epidermis' partition ratio and in a study of systemic measurement of topically applied CP in BA the cutaneous absorption seemed to be reduced.[3] Once the superficial ointment had been removed, the total CP concentration in the SC was higher for the PG than the BA formulation, which is consistent with this theory.

During the initial 8 h period, it may be presumed that the difference between the amount of CP applied and the sum of the amount in the gauze and the total amount in the SC, is the amount which has passed through the SC to the lower epidermis. The absorption from PG and from BA was less than 10% of the initial amount applied, and the greater absorption from PG than BA during these first 8 h although not statistically significant was again at least consistent with the theory of slower release from the BA base.

One further factor, not directly accounted for in the calculations given, is the separation of topically applied steroids into two functional components within the SC; one fraction is bound and takes no further part in the diffusion process, while the other mobile fraction diffuses.[4] However, once the bound component saturates the binding sites the presence of this component should not interfere with rate of transfer of steroid across the stratum corneum.

It would be helpful in interpreting the measurements of radioactivity in the outermost SSB ('A') if it were possible to distinguish between the component of drug which has persisted on the surface and the component which has been absorbed into the SC. One approach would be to wash the skin surface after removing the gauze at 8 h and to determine the radioactivity of the washings; the problem here, however, is that any washing method would be likely to remove the drug from within the very superficial SC.

The amount of radioactivity measured in the gauze swabs is surprisingly large, and almost 10 times the amount detectable in the SC at 8 h. The high proportion of steroid recovered from the dressing may result partially from too large a quantity of topical steroid being applied to the treated square. However, the demonstration that in an occluded site 80–90% of topically applied ointment is lost to the dressings is of importance when for instance considering optimal ointment strengths. If this figure holds true in the clinical situation, the bulk of topically applied ointment in clinical practice is probably lost. More of the BA formulation was detected in the gauze than the PG; possible explanations include a reduced rate of transfer of steroid across the SC in the former vehicle, or an increased affinity of this vehicle for the gauze swab.

The calculations given demonstrate that the multiple SSB technique can give kinetic information relating to stratum corneum penetration, and that this information can successfully allow for stratum corneum turnover.

It would be possible to extend the application of this technique and so make it more relevant to the real clinical situation. The technique could be used at earlier times after application, for example from 1 to 12 h. The possibility that the tracer kinetics might be altered by the real situation of regular ointment application could be overcome by treating the subject with a non-labelled preparation of the ointment before and after the radiotracer application.

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Topical prostaglandin E_2 in the treatment of chronic leg ulcers—a pilot study

G. ERIKSSON*, M. TORNGREN†, A. ALY‡ AND C. JOHANSSON†

*Department of Dermatology, Danderyd Hospital and Departments of †Internal Medicine and ‡Clinical Chemistry, Karolinska Hospital, Stockholm, Sweden

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SUMMARY

The results of treatment with topical PGE_2 dispersed in hydrocolloid granules, in nine patients with chronic leg ulcers is reported. The healing process was evaluated by stereophotogrammetry, which enables objective measurements of ulcer area and volume. Ulcers in eight patients healed completely and that in the ninth, almost completely. These results suggest that topical PGE_2 has a beneficial effect in the treatment of leg ulcers.

Parenteral administration of prostaglandins of the E and I series has been used in studies on the treatment of chronic ischaemic leg ulcers.[1–4] Accelerated healing has been attributed to vasodilatation and reduced thrombocyte aggregation,[5,6] but the impact of such transient phenomena on the healing process has not been established. Failure in some studies to demonstrate beneficial effects of prostaglandins[7] may be related to short treatment periods, the infusions were usually given over a 3-day period, and to insufficient bioavailability of the drugs at the ulcer site. In the human gastrointestinal tract, exogenous prostaglandins prevent mucosal damage and promote peptic ulcer healing.[8] The effect is probably local, since oral administration has invariably been more effective than parenteral when compared in animal models. This, together with the expected need to prolong treatment periods led us to carry out an open pilot study of the effect of topically applied natural PGE_2 on chronic leg ulcers. The successful outcome in the first two patients treated has been reported previously.[9] We present here the results in an additional nine non-diabetic patients.

METHODS

Patients

Nine patients, (five women and four men, mean age 74 years, range 56–86 years) were studied (Table 1). Two patients had combined arterial and venous ulcers (Patients A and B) and two (Patients C and D) had venous ulcers. Occlusive arterial disease was a causative factor in four patients (Patients E, F, G and H), and vasculitis in one patient with rheumatoid arthritis (Patient

TABLE 1. Clinical details of nine patients with chronic leg ulcers at the start of treatment with PGE_2

Patient	Sex	Age years	Ulcer type	Length of history (months)	Duration of present ulcer (months)	Hydrocolloid dressing treatment (months)	Effect	Comments
A	F	86	arterial + venous	66	24	> 12	none	...
B	M	56	arterial + venous	8	8	7	worse	...
C	F	73	venous	> 300	> 300	> 24	none	...
D	M	62	venous	300	8	4	worse	Cockett's operation on the same leg 12 years earlier
E	M	82	arterial	3	3	3	none	...
F	F	81	arterial	8	8	> 5	none	Amputation was planned at entry into study
G	M	79	arterial	1·5	1·5	10 days	worse	Pseudosarcoma Kaposi of the same leg 14 years earlier
H	F	74	arterial	10	10	1	none	Amputation of the other leg due to arterial obstruction 1 year earlier
I	F	64	vasculitis	240	240	7	none	Before and during study treatment with prednisolone and azathioprine

I). The diagnosis and degree of arterial insufficiency was established by measurement of segmental arterial pressure. None of the patients had clinical evidence of diabetes. The ulcers had been unaffected or had worsened during treatment with hydrocolloid dressings for periods of 10 days to more than 24 months (Table 1).

Procedure

Crystalline PGE_2 (kindly supplied by Upjohn Co. Ltd., Canada) was stored at −20°C. Appropriate amounts of PGE_2 were dissolved in 20 μl of ethanol and diluted with sterile isotonic saline to a concentration of 1–30 μg/ml. The final ethanol concentration was always below 1%. The prostaglandin E_2 solution was dispersed in hydrocolloid granules composed of gelatine, pectin, carboxymethylcellulose sodium and polyisobuthulene (DuoDERM®, kindly supplied by Squibb ConvaTec Inc., U.S.A.). PGE_2 was found to remain stable in the hydrocolloid granules at 37°C for more than 24 h as confirmed by thin layer chromatography of added ^{14}C-PGE_2. The ulcer was filled with the PGE_2 containing granules and then covered with an impermeable adhesive dressing (DuoDerm®). All treatments were started with PGE_2 solution at 1 μg/ml and, if tolerated, the concentration was subsequently increased. Dressings were changed two or three times weekly in the out-patient clinic at the same intervals as before entry into the study. The Ethical Committee of the Karolinska Hospital had approved the study and every patient gave informed consent.

Evaluation

Before start of the treatment and at regular intervals during the treatment period the area and volume of each ulcer was measured by stereophotogrammetry.[10] Bacteriological tests were performed at the start of the treatment and thereafter every second week. Clinical evaluation of the ulcers was made every week and side-effects recorded.

RESULTS

In eight of the nine patients the ulcers healed completely and in one patient almost completely. In four patients (Patients B, D, G and H) a transient increase of ulcer volume was observed at the start of treatment with PGE_2. After 4 weeks treatment with topical PGE_2 the mean ulcer volume was reduced to 49% of the original volume, after 8–10 weeks to 31%, after 12–14 weeks to 20% and after 16–18 weeks to 11%. The corresponding values for mean ulcer area were 75%, 47%, 38% and 31%. The course of healing was related to the length of history, ulcer size and the concentrations of PGE_2 solution applied (Table 2, Fig. 1).

The first two patients, Patients A and C, with combined arterial/venous and venous leg ulcers, respectively, were treated for a long period with a constant, low concentration of PGE_2 (2·6 μg/ml). A slow continuous reduction in ulcer size and volume was observed in Patient A during a treatment period of 15 weeks, after which the concentrations of PGE_2 were increased stepwise with accelerated healing which was eventually complete. In Patient C, treatment with 2·6 μg/ml PGE_2 was continued for 31 weeks. Initial progressive healing during the first 15 weeks with reduction of ulcer volume to 18% of the original volume was followed by deterioration during the 20th week with an increase in ulcer volume to 63% of the original size. Treatment with the same concentration of PGE_2 was continued for another 11 weeks during which time the ulcer failed to improve. Concentrations of PGE_2 were then increased to 4 μg/ml and further increased stepwise to 20 μg/ml at intervals of 1 to 4 weeks, which resulted in almost complete resolution of the ulcer.

In the remaining seven patients the concentrations of PGE_2 were increased at shorter intervals of 1 to 4 weeks up to a maximum concentration of 30 μg/ml. Complete healing within 8 weeks, on treatment with lower concentrations was observed in two patients with arterial ulcers (Patients E and G) and in one patient with a combined ulcer (Patient B) (Fig. 1).

TABLE 2. The effect of topical PGE_2 on ulcer volume

Patient	Ulcer volume at entry mm^3	Ulcer volume after topical PGE_2 % of original: 1 month	2–5 months	Final	Treatment period months
A	5446	67	53	0	10
B	401	32	0	0	2
C	1665	66	75	22	14
D	1125	83	59	0	12
E	943	22	0	0	1·5
F	578	20	24	0	11
G	205	0	0	0	1
H	243	110	0	0	3·5
I	1787	49	11	0	6

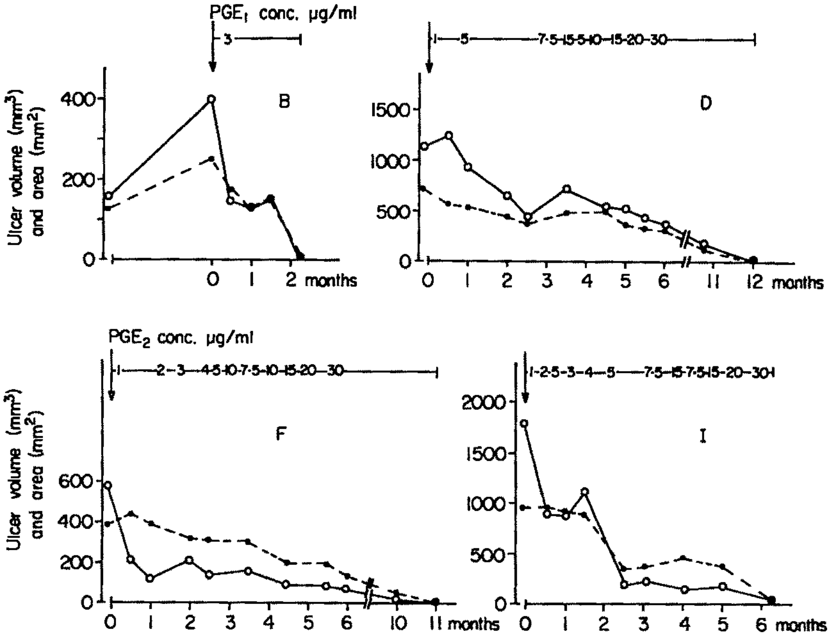

FIGURE 1. Healing of chronic leg ulcers, during topical treatment with different concentrations of PGE_2 in four patients (Patients B, D, F and I; Table 1). Stereophotogrammetry was used to assess ulcer volume (O——O) and area (●——●).

The patient who had been scheduled for amputation (Patient F) before treatment healed within 11 months on treatment with a maximal concentration of 30 μg/ml. A patient with an arterial ulcer on the foot (Patient H) healed within 14 weeks on treatment with a maximal concentration of 20 μg/ml.

Severe liposclerodermatotic changes of the skin surrounding the venous ulcer in Patient D (Fig. 1) probably contributed to the slow healing. After 24 weeks and a final PGE_2 concentration of 30 μg/ml the ulcer volume was still 33% of the original. However, after 1 year of treatment the ulcer was completely healed.

In the patient with a vasculitis ulcer (Patient I) (Fig. 1), who received the highest final PGE_2 concentration (30 μg/ml) the ulcer healed within 25 weeks.

During the healing process we observed that the start of treatment was followed by rapid clearance of debris covering the ulcer; granulation tissue started to appear at the bottom of the ulcer with subsequent development of islands of skin within the ulcer and a slow growth inwards from the edges. There were no signs of irritation of the skin surrounding the ulcer. The bacteriological results did not differ from those in earlier trials.[11,12] In general, the ulcers kept their initial flora throughout the treatment period.

The only recorded side-effect was local pain, which occurred in eight patients, coinciding with the first application of a more potent PGE_2 solution. The pain started 1–4 h after the application and lasted up to 14 h. When pain occurred, concentrations of PGE_2 were reduced with subsequent smaller increases over a longer period of time. In three patients (Patients D, F and I) these reductions in dose were accompanied by a transient increase in ulcer size, but healing was resumed when higher concentrations could be tolerated by the patient.

DISCUSSION

The healing of chronic leg ulcers on treatment with topically applied natural PGE_2 has now been observed in nine further patients, in addition to the two already reported.[9] All patients had been treated previously without success with hydrocolloid granules and dressings alone, a treatment which has been reported to enhance healing of chronic skin ulcers and does not cause allergic reactions.[11–14] The treatment and follow-up of the patients during the study differed from the prestudy period only with respect to the addition of PGE_2 to the hydrocolloid granules. We therefore, conclude that topical PGE_2 contributed to the favourable outcome. We also conclude that the concentration of PGE_2 should be progressively increased and maintained at the maximal tolerable level in order to shorten the healing time. As this became apparent during the study, the healing process in the two patients already reported and in an additional two of the nine patients reported here might have been unnecessarily delayed.

There may be several reasons for the need to increase the concentration of PGE_2 during the healing process. When the concentration of PGE_2 is kept constant and the ulcer volume decreases the dose applied will actually diminish during treatment. Thus not only the concentration, but the total applied dose of PGE_2 may be important in accelerating healing. Alternatively, tolerance to PGE_2 might develop, with a subsequent need for increased concentrations.

Prostaglandin concentrations should be low when treatment is started in order to avoid pain. In the present study the usual initial dose of PGE_2, 1 μg/ml, equals the concentration used for intravascular administration and was tolerated by all patients when applied topically dispersed in hydrocolloid granules. At a higher initial concentration, 10 μg/ml, which did not irritate normal skin, intolerable pain occurred in one patient.[9] On the other hand this dose and higher concentrations up to 30 μg/ml can be applied without discomfort if doses are started low and elevated successively at suitable intervals.

Following topical application of increasing concentrations of PGE_2, the healing process followed a normal course. Initially the rapid disappearance of ulcer debris was sometimes associated with an apparent transient increase of ulcer volume. Thereafter the healing process started with development of granulation tissue and inward growth of the ulcer margins. In some cases epithelial islands appeared within the ulcer. The different rates at which ulcer volume and area diminished emphasize the importance of evaluating both parameters in pilot treatment studies. Repeated sampling did not demonstrate any detrimental effects of topical PGE_2 on the bacterial flora.

No attempts were made to measure the bioavailability of PGE_2 from the hydrocolloid. However, the test of chemical stability of PGE_2 in the hydrocolloid as well as the outcome of the study including the side effects (pain at the start of the treatment) indicate the presence and effect of an active substance.

A local irritant effect of PGE_2 as the trigger to accelerated healing of chronic leg ulcers cannot be excluded, but this seems unlikely because there was no irritation of the surrounding skin. For the same reason vasodilatation can hardly account for the whole effect, although the importance of local blood flow in distributing oxygen and nutrients to the ulcer area was not assessed in the present study.

As expected, the larger and, in particular, the deeper ulcers healed more slowly than the small ones. More important than this variation, however, is the fact that complete or nearly complete healing of the chronic ulcers occurred irrespective of their pathogenesis. This suggests that common local mechanisms are triggered by the applied PGE_2 and contribute to healing. In the gastrointestinal tract the capacity of certain prostaglandins to prevent mucosal damage and accelerate peptic ulcer healing has been associated with their stimulation of mucosal defence and repair factors.[8] In addition, orally administered prostaglandins of the E series have a trophic action on intestinal epithelia by reducing luminal losses of cells and slowing their migration.[15] The importance of such properties in promoting healing of chronic skin ulcers is still speculative. The results of the present study suggest that topical treatment with PGE_2 or analogues may provide a useful and inexpensive alternative to accelerate healing of chronic leg ulcers. Controlled studies are now required to confirm this.

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Monochloroacetic acid and 60% salicylic acid as a treatment for simple plantar warts: effectiveness and mode of action

K.STEELE*, P.SHIRODARIA.§ M.O'HARE,* J.D.MERRETT,† W.G. IRWIN*, D.I.H. SIMPSON§ AND H.PFISTER‡

*Department of General Practice, Dunluce Health Centre, Belfast, § Department of Microbiology and Immunology and † Department of Medical Statistics, The Queen's University of Belfast, Northern Ireland and ‡ Institute of Virology, Erlangen-Nurnberg, West Germany

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SUMMARY

Monochloroacetic acid crystals and 60% salicylic acid ointment was found to be more effective than placebo as a treatment for simple plantar warts in a double blind study on 57 patients. Nineteen (66%) patients in the active treatment group compared with five (18%) patients in the placebo group were cured after 6 weeks ($P=0{\cdot}002$). The active treatment was associated with a significantly higher cure rate 6 months after entry ($P=0{\cdot}04$). Treatments were well tolerated. IgG or IgM antibodies or both to human papilloma virus (HPV) types 1 or 2 or both were detected significantly more frequently in the actively treated group 6 weeks after entry ($P=0{\cdot}0005$). Twelve (50%) patients considered to be cured had no detectable secondary immune response. Our results suggest that cure does not depend primarily on the humoral system but rather on mechanical destruction of wart tissue, or occurs as a result of cell mediated immunity.

Monochloroacetic acid is a powerful irritant which hydrolyses protein with resultant inflammation and cellular destruction. This escharotic is not tissue specific, can be applied as a crystal or solution and great care must be taken of the surrounding skin during its application.[1] Salicylic acid is the most commonly used agent in preparations prescribed for plantar warts. It is present in varying strengths from 11–25% in the proprietary preparations Cuplex,® Duofilm,® Salactol® and Posalfilin.® Huber and Christophers[2] using a fluorescence staining method concluded that the drug destroyed intercellular cohesiveness allowing stratum corneum cells to separate off.

Dagnall[3] described the use of monochloroacetic acid solution for a period of 72 h followed by

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Correspondence: Dr K. Steele, Department of General Practice, Dunluce Health Centre, 1 Dunluce Avenue, Belfast BT9 7HR, U.K.

debridement in 300 patients with plantar warts. He claimed a success rate of more than 90% using repeated treatments. Dutta[4] advocated the use of monochloroacetic acid crystals for a period of 48 h followed by debridement. In this series of 48 patients, 66% achieved a cure. A comparative study of monochloroacetic acid has not been described to our knowledge. This is a popular treatment in conjunction with 60% salicylic acid ointment in chiropody circles in Northern Ireland.

A relationship between treatment with wart ointments and humoral provocation was suggested by McConahy.[5] He reported only eight recurrences in 150 patients cured with ointment. He related this finding to a protective humoral response induced by persistent irritation by the ointment. Shirodaria[6] using indirect immunofluorescence techniques demonstrated IgG antibody in all patients tested who had regressing warts. Whether this was a cause or effect of regression, or a result of treatment it could not be demonstrated.

Morrison,[7] on the other hand, using a leukocyte migration inhibition test to study sera from 14 patients treated with ointment or cryosurgery, claimed that there was evidence to suggest that cell mediated immunity increased at the time of wart resolution.

This theory was supported by Tagami and Takigawa[8] who demonstrated mononuclear cell infiltrates in plane warts prior to resolution. Thivolet *et al*[9] using intradermal injections of formalin treated HPV to provoke a cell mediated immune response showed that patients with concurrent or recent history of HPV infection were significantly more likely to mount a response to the injection.

The aims of the present study were, first, to compare monochloroacetic acid crystals and 60% salicylic acid ointment with placebo with respect to effectiveness and patient tolerance in the treatment of simple plantar warts, and second to determine if either treatment or cure of this condition was associated with a humoral immune response. Type specific antigens (HPV1 and 2) in biopsy specimens were used to detect type specific IgM and IgG antibody by means of an indirect immunofluorescence test.

METHODS

Fifty seven patients were recruited from the wart clinic at Dunluce Health Centre and all gave informed consent. Patients under 7 years of age, and patients with mosaic or mother and daughter types of plantar wart or with warts on other parts of the body were excluded from the trial. Patients whose plantar warts were more than 1cm in diameter, who had more than three simple plantar warts, or who had received another wart treatment within the previous month were also excluded. The active preparation consisted of monochloroacetic acid crystals and 60% salicylic acid ointment. Sodium sulphate crystals and emulsifying ointment were used as placebo. Because monochloroacetic acid has an odour of vinegar, containers containing placebo were dabbed with acetic acid to prevent recognition of the active preparation by smell. Preparations were made up, dispensed and allocated using a random numbers table, by the health centre pharmacist.

Skin surrounding the plantar wart or warts was protected with 95% silver nitrate and masked with zinc oxide tape. An aperture pad of semi-compressed felt was used to hold the crystal against the plantar wart. The latter was cemented in with ointment. The whole dressing was fixed in position with Mefix tape. Patients were instructed to keep the dressing dry and to inform the chiropodist if paracetamol failed to relieve any subsequent pain. Dressings were left in place for 1 week. The resulting eschar was then pared and debrided. No further dressings were applied, and the warts were reviewed at 3 and 6 weeks when the final result was noted. A cure was taken as the return of normal skin creases[10] and assessment was made by an independent observer.

Patients were questioned about pain from the plantar wart at entry, as a result of the 1st week's treatment, and after 6 weeks. Patients were re-examined 6 months after entry to assess the long-term outcome of treatment. Sixteen untreated plantar warts were excised under local anaesthetic, snap frozen in liquid nitrogen and stored at −70°C. The lesions were sectioned at 5μm on a Brights cryostat.

Nine unfixed frozen sections from each biopsy were covered with 50μl lysis buffer (10mM Tris HCl pH 7·5, 10mM EDTA, 0·1M NaCl, 0·5% SDS) and incubated for 1 h at 37°C in a moist chamber. The lysates were pooled into one Eppendorf tube and digested with proteinase K (500 μg/ml) at 37°C for 30 min. Following RNase A treatment (50 μg/ml, for 5 min at room temperature) the DNA was extracted twice with ether. The DNA was precipitated with ethanol, digested with *Bam* HI and subjected to gel electrophoresis. Southern blots were prepared on nylon membranes (Gene Screen®) according to the recommendations of the manufacturer.

Reference DNAs for HPV 1 (the identity of the DNA, which was cloned from a plantar wart, was demonstrated by physical mapping), and HPV 2[11] were labelled by nick translation, using radioactive phosphorus, to a specific activity of about 10^8 cpm/μg DNA. The filters were pre-incubated with double concentrated SSC buffer (0·15M NaCl, 15 mM sodium citrate), 0·1% Denhardt solution and 100 μg/ml yeast RNA for 3 h at 65°C. Hybridization with probe DNA (50,000 cpm/cm^2 filter) occurred in the presence of 50% formamide, 5 times concentrated SSC buffer, 80mM sodium phosphate buffer (pH 6·2), 100 μg/ml yeast RNA, 0·02% Denhardt solution and 0·1% SDS for 2 days at 37°C. Filters were washed as described by Pfister.[12]

Blood samples (5ml) were taken from each patient at entry and after 6 weeks and, using type specific antigen as determined above and an indirect immunofluorescence test as previously described[6] each sample was tested for HPV 1 IgM, HPV 1 IgG, HPV 2 IgM and HPV 2 IgG. Group means were compared using Student's *t*-test, but because of possible distribution problems this was supported by the non parametric Mann Whitney U test. The sex ratios in the two groups, and the proportions of patients with particular numbers, duration and sites of warts and the proportions experiencing pain were, compared using a chi-squared test.

RESULTS

All 57 patients completed the trial. Patient details are given in Table 1. The mean age of patients in the active treatment group was 18 years (range 7–30 years) and that in the placebo group 12 years (range 8–34 years). There were no significant differences between the active and placebo groups in the mean age of the patients, the sex ratio, number of plantar warts per patient, duration of plantar warts prior to entry, use of self-medication prior to entry and site of plantar warts. Nineteen (66%) of the active treatment group compared with five (18%) of the placebo group were cured after 6 weeks ($P=0·002$). Twenty-four (83%) of the active group compared with 15 (54%) of the placebo group were cured 6 months after entry ($P=0·04$). Forty two (74%) patients complained of pain from their plantar wart prior to entry. There was no significant difference between the numbers of these patients in the two groups. During the first week's treatment, 23 (80%) of the actively treated group compared with 12 (43%) of the placebo group complained of pain ($P=0·02$). One patient developed a cellulitis secondary to treatment, but this responded rapidly to antibiotics. During the final week of treatment one (4%) of the actively treated group compared with seven (35%) of the placebo group complained of pain from their plantar warts ($P=0·04$).

TABLE 1. Details of 57 patients with plantar warts who took part in the trial

	No. of patients (%)	
	Active treatment group ($n = 29$)	Placebo group ($n = 28$)
Sex Male	13 (45)	13 (46)
Female	16 (55)	15 (54)
No. of plantar warts		
Single	17 (59)	18 (64)
Multiple (2 or 3)	12 (41)	10 (46)
Duration of plantar wart		
Less than 3 months	7 (24)	11 (39)
3–5 months	11 (38)	8 (29)
6–11 months	3 (10)	3 (11)
12–23 months	5 (17)	4 (14)
24 months or more	3 (10)	2 (7)
Self-medication used prior to entry		
Yes	16 (55)	13 (46)
No	13 (45)	15 (54)
Site of warts		
Forefoot	15 (52)	16 (57)
Midfoot	1 (3)	2 (7)
Heel	9 (31)	7 (25)
Two or more sites	4 (14)	3 (11)

Braces indicate numbers grouped for purposes of statistical analysis

The numbers of patients in each group who showed an antibody rise subsequent to treatment are given in Table 2. An antibody rise means the detection of IgM or IgG antibodies or both to HPV types 1 or 2 or both at 6 weeks when these had not been detected at entry. Twenty (69%) of the actively treated group compared with eight (29%) of the placebo group were shown to have an antibody rise ($P = 0{\cdot}0005$). The outcome of treatment in relation to the presence or absence of an HPV 1 or 2 IgG immune response as a result of treatment in the two groups is shown in Table 3. A secondary immune response was detected in both the placebo and the active groups. It was detected both in patients who had been cured and patients who had failed to respond to treatment. We were unable to detect the development of a secondary immune response in twelve (50%) patients responding to treatment.

DISCUSSION

Our findings confirm those of Dutta[4] and show that monochloroacetic acid/60% salicylic acid is an effective treatment for simple plantar warts. The need for a placebo group was shown by the high placebo cure rate (18%). This is probably higher than we might have expected from natural regression and may be related to the regular paring of lesions carried out in both treatment groups by the chiropodist. We were not able to achieve the 90% cure rate claimed by Dagnall.[3]

TABLE 2. Occurrence of an antibody rise* in patients in the actively treated and placebo groups

	Active (*n*=29)	Placebo (*n*=28)
Antibody rise with treatment	No. of patients (%)	No. of patients (%)
Detected	20 (69)	8 (29)
Not detected	9 (31)	20 (71)

* An antibody rise means the detection of IgM or IgG or both to HPV types 1 or 2 or both at 6 weeks when this had not been detected at entry.

TABLE 3. Presence of IgG in relation to the outcome of treatment in the actively treated and placebo groups

	No. of patients			
Treatment Group	Cured IgG present	Cured No IgG present	Treatment IgG present	Treatment No IgG present
Placebo	2	3	4	19
Active	10	9	1	9

This is probably related to differences in treatment techniques, as this author used monochloroacetic acid solution and repeated treatments. We felt justified in excluding patients with plantar warts larger than 1cm as Rasmussen[13] had shown that more than 70% of plantar warts in his survey were between 2·4 and 8·1mm in diameter. Approximately 50% of patients had used self-medication prior to entry. This reflects the poor results of self treatment for this condition. Six months after entry there was still a significant difference in the cure rate between the treatment groups in favour of the active group. This would imply that although natural regression increased with time (54% at 6 months) active therapy is still justified in terms of cure rate alone.

The treatments were well tolerated and no patient withdrew. Seventy four percent of patients complained that their plantar warts were painful prior to entry and these patients were equally distributed between treatments groups. Significantly more patients in the actively treated group than in the placebo group complained of pain during the first week of treatment. During the final week of treatment significantly more patients in the placebo group complained of pain. Our explanation for these findings is that pain secondary to active treatment only lasted a few days and that during the final week's treatment significantly more patients in the placebo group had failed to respond to treatment and as a result continued to complain of pain from their plantar warts.

Thirty four patients (60%) had HPV 1 IgM antibody in their sera on entry to the trial. This confirmed the association of plantar warts with HPV 1 shown in previous biopsy series.[14,15] Only five (9%) had HPV 2 IgM antibody. Only one patient had HPV 1 IgG on entry. This confirmed earlier work[16] which showed that IgG antibody was rarely found in non regressing lesions. Almedia[17] postulated that the release of antigen from cutaneous warts in their natural state is not sufficient to provoke a secondary (IgG) immune response.

Our results show that active treatment provoked a humoral immune response (a rise in IgM or IgG or both) in significantly more patients than did placebo. Thus, monochloroacetic and salicylic acid are much more likely to break down and destroy virus containing cells than is the mechanical irritation alone, administered to the placebo group. This would result in a greater exposure of the virus to the immune system with an increased humoral response. Although there was a trend for a secondary immune response (an increase in IgG only) to be more frequent in the active group this was not statistically significant. An IgG response requires a greater antigenic load to be exposed to the circulation. This did not occur to a significant extent probably because of the natural isolation of the lesions from the immune system.

Shirodaria[14] showed that HPV IgG antibody was associated with naturally regressing warts in 97% of cases, suggesting a possible role for the humoral immune system in natural regression. Although we detected IgG in the sera of 12 patients who were cured (10 active and two placebo) we failed to detect IgG in the sera of 12 other patients who were also cured (nine active and three placebo). These results would suggest that cure does not depend primarily on the humoral system, but rather on chemical or mechanical destruction of wart virus infected tissue or that it occurs as a result of cell mediated immunity undetectable by the methods used. In five patients (four placebo and one active) IgG was detected in their sera at 6 weeks and they failed to respond to treatment. Only one of these patients was cured 6 months after entry. This finding would also militate against lgG promoting resolution.

Our results are not in agreement with the longitudinal study by Pyrhonen and Pentlinen[18] who found that IgG was associated with a cure in 94% of patients with cutaneous warts. Five (29%) of our patients with IgG were not cured. We would disagree with Morison[7] who, using the results of a longitudinal study, claimed that IgG was unlikely to be a side effect of treatment. Our results show that monochloroacetic acid and 60% salicylic acid is more effective than placebo in the treatment of simple plantar warts, and support our hypothesis that the active treatment is more likely, secondary to cellular destruction, to expose HPV to the humoral immune system with resultant humoral provocation. We have also shown that an antibody rise is not necessary for a cure, nor does it ensure a cure. We would suggest that mechanical factors or cell mediated immune responses, or both, play a part in the cure of these lesions.

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Drug-induced pseudolymphomatous skin reactions

S.H.KARDAUN, E.SCHEFFER* AND B.J.VERMEER†

Department of Dermatology, State University Hospital, Groningen and Departments of Pathology* and Dermatology†, State University Hospital, Leiden, The Netherlands

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SUMMARY

We present two patients with generalized and three with localized skin eruptions due to various non-anticonvulsant drugs, with a histological picture of a pseudolymphoma of the skin. Cessation of the causative drugs resulted in disappearance of the lesions in all cases.

The pseudolymphoma syndrome has been described as a hypersensitivity reaction to anticonvulsant drugs.[1–6] Its pathogenesis is unknown. It consists of the triad of fever, generalized rash and lymphadenopathy. In some cases, varying degrees of malaise, hepatosplenomegaly, abnormal liver function tests, arthralgias, eosinophilia and blood dyscrasias may be present.[4]

If the syndrome is not recognised and the causative agent not discontinued, unnecessary anti-tumour therapy may be prescribed.[1,5,7] This can lead to a fatal outcome.[7] Also, complications of the syndrome itself may lead to life-threatening hypersensitivity reactions with extensive skin lesions, and serious organ involvement including fatal hepatitis,[9] agranulocytosis[10] or pancytopenia.[11] The syndrome may be confused with severe infectious exanthemas.[8,12,13] Although in most cases regression occurs soon after discontinuing the drug responsible, this is not always observed and in some cases a true lymphoma develops.[2,3,14–16] The clinical diagnosis of pseudolymphoma in cases reported in the literature was not always confirmed histologically. Histological investigation of the involved skin has only been reported in a few cases; it may be non-specific,[7,17] and include a cutaneous vasculitis.[18] Three reports however, mention a histological picture resembling mycosis fungoides.[5,19,20]

We have carried out a prospective histological study of 80 patients with drug-induced skin lesions. We report here five patients in whom the histological findings aroused suspicion of a lymphoma.

CASE REPORTS

Case 1

A 56-year-old man with cardiac problems developed a generalized, progressive pruritic rash, consisting of erythemato-squamo-papular, partly urticarial, lesions 4 weeks after starting

Correspondence: S.H.Kardaun, Department of Dermatology, University Hospital, P.O. Box 30.001, 9700 RB Groningen, The Netherlands.

mexiletine chloride 200 mg per day (an anti-arrhythmic). In addition, he had oedema and scaling of the face, hyperkeratotic palms, palpable lymph nodes in the groins and cervical region and conjunctivitis. Discontinuation of the drug for 2 days resulted in a regression of the lesions. Because of cardiac problems the drug had to be reinstituted; one i.v. dose (1000 mg) resulted in aggravation of the skin lesions. After cessation of the drug, complete clearance occurred within about 2 weeks. Laboratory investigations revealed a transient eosinophilia and leukocytosis. Histologically, the skin showed focal parakeratosis, acanthosis, a dense band-like dermal infiltrate, consisting of lymphoid cells, many histiocytes, eosinophils and a few mitoses. There was endothelial cell swelling, vascular proliferation, extravasation of erythrocytes, and epidermotropism of large cerebriform mononuclear cells, lymphocytes and eosinophils with spongiosis. Lymph node cytology did not reveal signs of malignancy.

Case 2

For more than 3 years, a 41-year-old lady had suffered from itchy, slightly infiltrated erythemato-papular lesions in the face, exacerbating after sun exposure (Fig. 1). Because of a psychiatric disorder she had been taking thioridazine retard for $5\frac{1}{2}$ years. Four weeks after discontinuing the drug, all lesions disappeared. Photopatch testing was positive with thioridazine 1%, chlorpromazine hydrochloride 1% and promethazine chloride 2% (all in petrolatum).

Routine laboratory results were normal or negative. Histologically, the skin revealed parakeratosis, dense, sharply demarcated, perivascular, atypical lymphoreticular infiltrates with some blast-like cells, plasma cells, histiocytes, and a few eosinophils and neutrophils with little infiltration of the basal layer of the hair follicles and some dermal elastosis (Figs. 2 and 3).

Case 3

A 38-year-old man with seropositive rheumatoid arthritis, who was taking several anti-rheumatics, developed a generalized pruritic eczematous reaction, consisting of maculo-

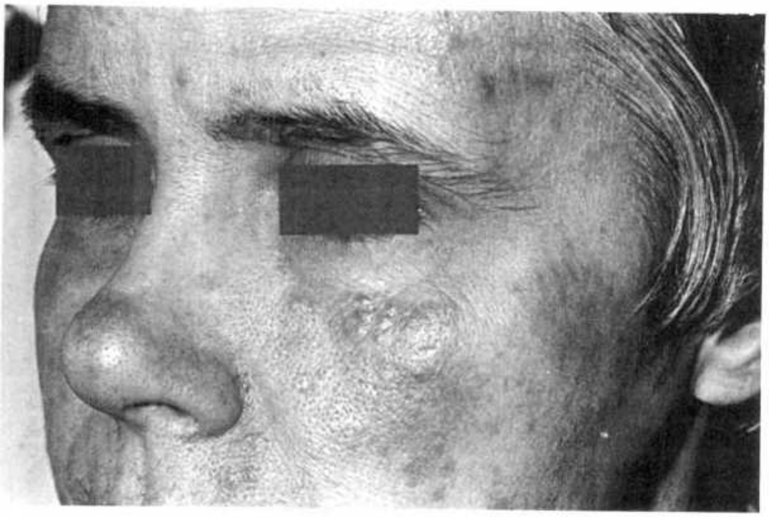

FIGURE 1. Case 2. Slightly infiltrated erythemato-papular lesions in the face.

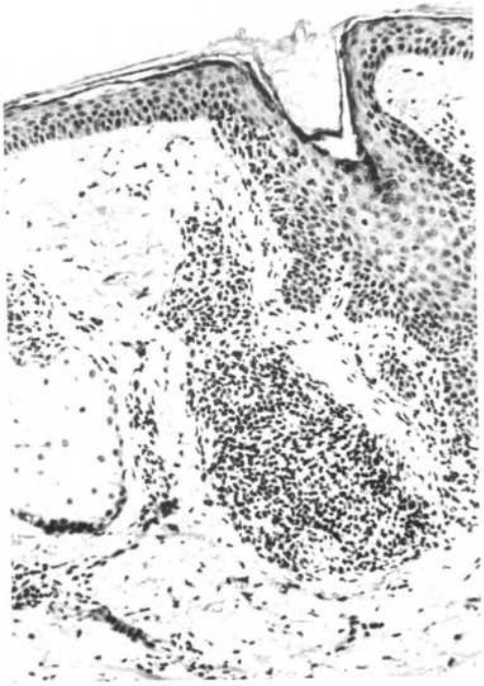

FIGURE 2. Case 2. Sharply demarcated atypical lymphoreticular perivascular infiltrate. (Original × 100).

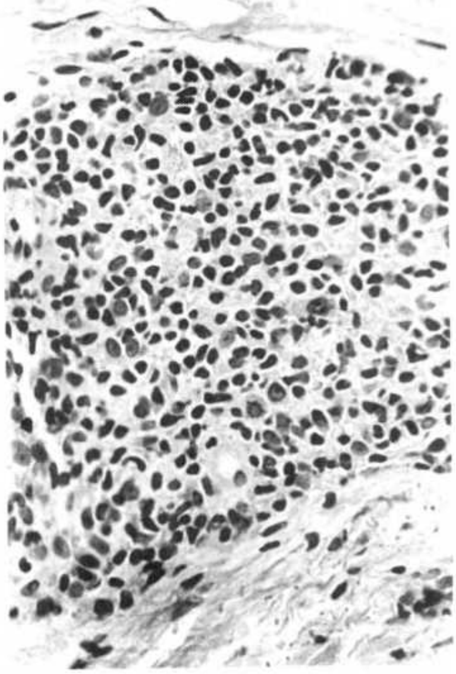

FIGURE 3. Case 2. Dermal infiltrate showing several blasts, plasma cells, histiocytes and mitoses. (Original × 400).

papular, partly erosive lesions and crusts. Eight months before the eruption commenced, he had started to take D-penicillamine. Laboratory investigations showed transient leukocytosis, haematuria and very slight albuminuria. Five weeks after discontinuing D-penicillamine, the symptoms gradually disappeared. Histologically, the epidermis showed crusts, acanthosis, hyperkeratosis, and Pautrier's micro-abscess-like structures with histiocytes and cerebriform mononuclear cells with slight spongiosis in the adjacent epidermis. The dermal infiltrate contained cerebriform mononuclear cells and many eosinophils. Some cerebriform mononuclear cells, eosinophils and lymphocytes were also present in the epidermis.

Case 4

A 52-year-old lady had suffered periodically for 4 years from itchy, eczematous papules and plaques, mainly localized to the hands, arms and legs, but also present on the trunk. She also had conjunctivitis. On each occasion the eruption returned after she had taken one to two tablets Soneryl (butobarbital) and Triatussic bitabs (containing paracetamol, noscapine and pheniramine) for a common cold. After discontinuation of these drugs, the lesions faded within a few weeks, leaving some hyperpigmented macules. In the course of time the eruptions became worse. For more than a year they had been vesicular and were accompanied by fever. Once the same lesions had appeared after taking Triatussic alone. After discontinuing the drugs the lesions did not return. Routine laboratory investigations revealed no abnormalities. Histologically, the epidermis showed some acanthosis, focal infiltration of lymphoid cells including large cerebriform mononuclear cells, spongiosis, and individual necrotic keratinocytes. In the dermis, perivascular infiltrates were observed consisting of atypical lymphoreticular cells with a few mitoses and an occasional eosinophil; focal extravasations of erythrocytes and dermal oedema were present.

Case 5

A 77-year-old woman suffered from arterial insufficiency and intermittent claudication of the right leg, for which she underwent partial amputation. She took paracetamol for 8 weeks and carbamazepine for 6 days for the pain. In addition she had been taking labetalol for 7 months, and Moduretic (amiloride and hydrochlorothiazide) for 3 years for hypertension. She developed a pruritic indurated, erythematous 6 × 7 cm plaque on the left forearm, with a slightly papulovesicular centre (Fig. 4). A fixed drug eruption, probably due to paracetamol, was suspected and the drug was discontinued. However, within 1 week the lesion enlarged with central necrosis and bulla formation, and a second, nummular, pink, cocarde-like lesion appeared on the left leg. Moduretic was then replaced by Lasix (frusemide). Two weeks later, the lesions had healed. Systemic symptoms were absent. Routine laboratory investigations revealed no abnormalities. Histological investigation of the skin showed a moderate, perivascular and periadnexal infiltrate, consisting of lymphoid cells, occasional cerebriform mononuclear cells, many eosinophils, several histiocytes, and a few blast-like cells (Fig. 5) and neutrophils, with some involvement of hair follicles. There was epidermotropism with intraepithelial and subcorneal Pautrier's micro-abscess-like structures filled with histiocytes, cerebriform mononuclear cells and some eosinophils with spongiosis in the adjacent epidermis (Fig. 6).

DISCUSSION

The pseudolymphomas or lymphomatoid reactions of the skin form part of a group of reactive conditions. Clinically or histologically or both, they are more or less similar to malignant

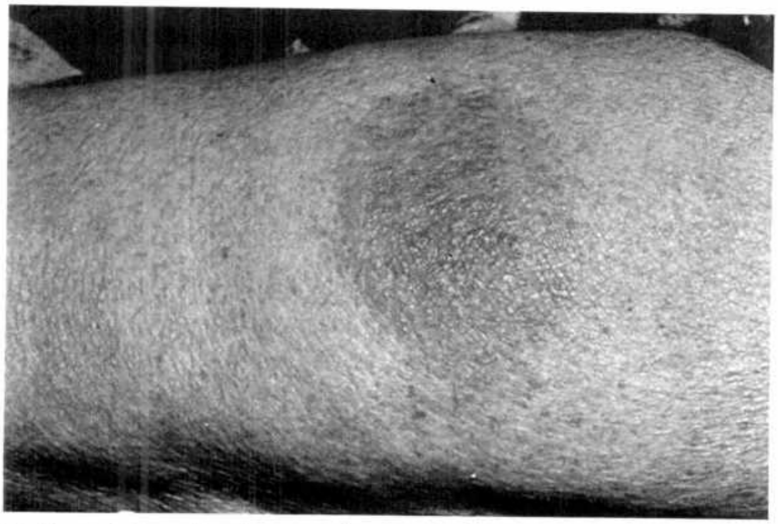

FIGURE 4. Case 5. Indurated erythematous plaque on the forearm with central papulovesiculation.

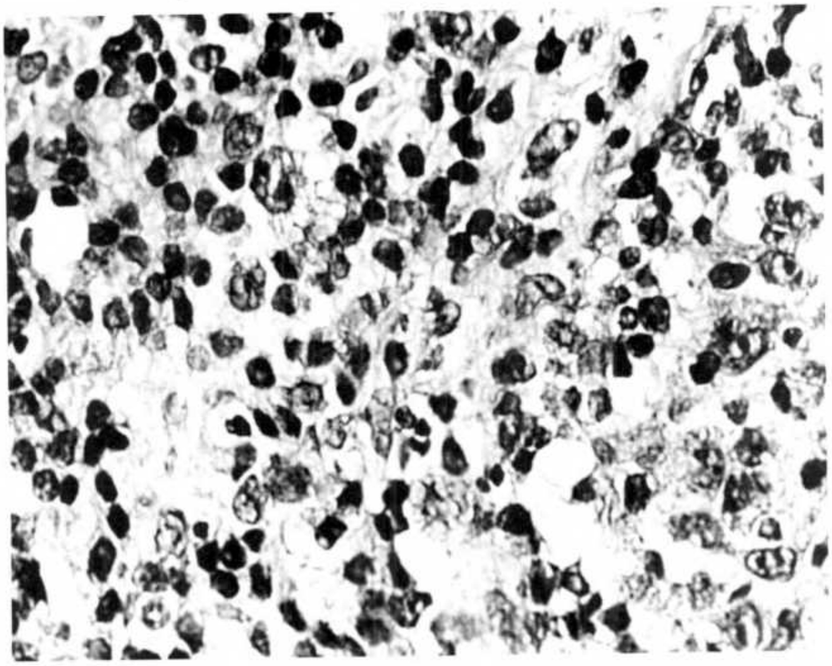

FIGURE 5. Case 5. Dermal infiltrate showing histiocytes, cerebriform mononuclear cells and blasts. (Original × 400).

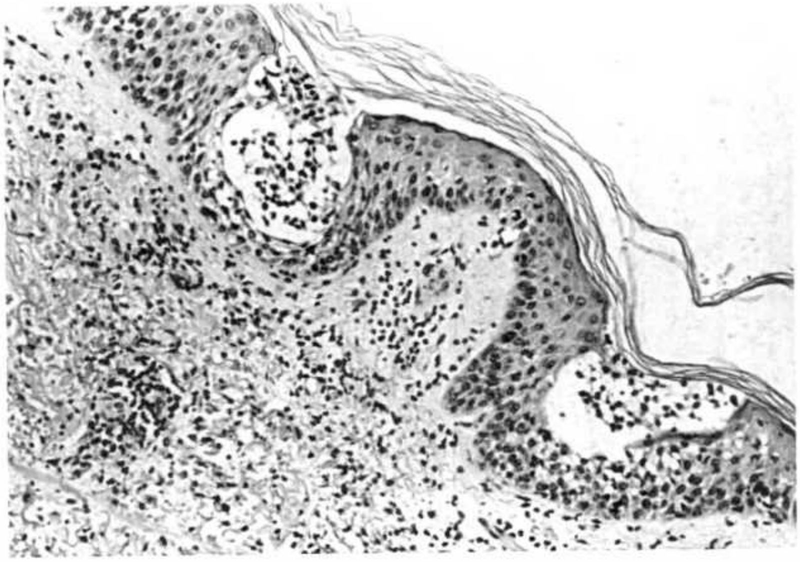

FIGURE 6. Case 5. Pautrier's micro-abscess-like structures and epidermotropism with histiocytes, cerebriform mononuclear cells and eosinophils. (Original × 100).

cutaneous lymphoma and are characterized by infiltration and proliferation of T or B lymphocytes or both in the skin. Histological differentiation from malignant lymphoma may be very difficult.

Among the lymphomatoid reactions are those with mycosis fungoides-like patterns.[19] Lymphomatoid reactions characteristically may show blast-like cells, cerebriform mononuclear cells with large nuclei, and Pautrier's micro-abscess-like structures. Blast-like cells and large cerebriform mononuclear cells may also be encountered in reactive processes.[21] The Pautrier's micro-abscess-like structures in our cases differed from the Pautrier's micro-abscesses found in mycosis fungoides or Sézary syndrome in being mainly composed of histiocytes.

Moreover, the adjacent epidermis showed spongiosis, as seen in reactive processes.[22] Also, in our cases we saw numerous eosinophils in the epidermis, sometimes even in the Pautrier's micro-abscess-like structures. In our experience, Pautrier's micro-abscess-like structures which contain considerable numbers of histiocytes may be seen in hypersensitivity reactions, for example contact dermatitis, whereas in mycosis fungoides and Sézary syndrome the Pautrier's micro-abscesses mainly consist of large, cerebriform mononuclear cells, or blast-like cells or both.

In our opinion, Cases 1, 3, 4 and 5 suffered from the pseudo mycosis fungoides syndrome (PMFS), as described by Rosenthal *et al.*[19] Although in Cases 3, 4 and 5 the syndrome was not fully developed, histologically, the skin lesions in these four patients were suspected to be a T cell lymphoma. Case 5 showed clinical and histological similarities to a case presented by Wolf, Kahane and Sandbank.[20] These authors considered the term PMFS appropriate, also when, in pseudolymphoma syndrome, there are localized, erythematous plaques instead of an exfoliative dermatitis. From the scarce reports, it might be concluded that this PMFS, as a subtype of the pseudolymphoma syndrome, is a rare event. However, cases may go unreported since skin biopsies are seldom performed in the pseudolymphoma syndrome.

The biopsy from a skin lesion in Case 2 mainly differed from the other cases in that epidermotropism was lacking. The term PMFS does not seem appropriate for this patient.

A striking fact in most of our patients was that they, like the cases described in some other reports,[19,20,23] had used the offending drugs for several months or years before the onset of the eruption. According to the literature, in the majority of cases the pseudolymphoma syndrome manifests itself after 2 to 8 weeks of diphenylhydantoin therapy,[5,6,16] whereas the development of a true lymphoma requires a period of many years of drug therapy.[15,24]

Most anticonvulsant drugs can produce the pseudolymphoma syndrome. The most common offenders are diphenylhydantoin (Dilantin), mephytoin (Mesantoin) and also trimethadion (Tridione).[5] Cross-reactions occur most frequently among the hydantoin type of drugs and in the phenobarbital group.[5,13]

Despite the fact that all hydantoins may produce lymphadenopathy, only phenytoin has been associated with true lymphoma. Anthony,[24] in a retrospective study, demonstrated a 10-fold increase in the incidence of lymphoma in patients on long-term diphenylhydantoin compared with a control group. In mice, some strains showed malignant transformation after having been fed with diphenylhydantoin, while in other strains such changes were not found.[25] On the basis of these data, a genetic predisposition to malignant transformation due to hydantoin may be postulated.[19] Before true lymphoma develops, a state of pseudolymphoma may exist, which represents lymphoid hyperplasia and thus may be a premalignant state.[15]

In 4 out of 5 cases reported here, only skin lesions were present, localized in Cases 2, 4 and 5 and generalized in Case 3. Case 1, with a generalized skin eruption, also developed palpable lymph nodes. Contrary to most published reports, the offending drugs were not anticonvulsants, except perhaps in Case 4, who had also taken Soneryl. Involution of the skin lesions after cessation of the suspected drugs strongly suggests a drug-induced reaction. The positive photopatch tests in Case 2 and the positive reactions on rechallenge in Cases 1 and 4, support a drug-induced aetiology. At present it appears to be unlikely that any of these cases will evolve into a malignancy, since none of the patients had relapsed after follow-up periods of between $2\frac{1}{2}$ and $4\frac{1}{2}$ years.

Causes of lymphomatoid reactions often remain obscure. Drug reactions should be seriously taken into consideration, even if the drugs are not anticonvulsants or the pseudolymphoma syndrome has not developed fully. Clinically, these cases are heterogeneous in showing different skin lesions; histologically they may present a pseudolymphomatous, or even a 'pseudo mycosis fungoides' picture. Both the clinician and the pathologist should be familiar with this condition; elimination of the drug responsible will rapidly solve the problem.

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Cutaneous presentation of prolymphocytic leukaemia

R.A. LOGAN AND N.P. SMITH

St John's Hospital for Diseases of the Skin, 5 Lisle Street, London WC2H 7BJ, U.K.

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SUMMARY

We report a case of prolymphocytic leukaemia which presented with annular, purpuric, erythematous plaques in the skin. Histology of the lesions showed cutaneous invasion with leukaemic cells. The lesions cleared with chlorambucil and prednisolone, and the leukaemic state responded to deoxycoformycin, an experimental anti T cell agent.

CASE REPORT

History

A 61-year-old, Caucasian man presented to St John's Hospital for Diseases of the Skin, London, in April 1986, with an 8-week history of non-itchy skin lesions on the head, neck and lumbar region associated with painful mouth ulcers. The mouth ulcers improved following treatment with hydrocortisone lozenges (Corlan®). However, the skin lesions spread in a centrifugal fashion despite treatment with topical steroids, a topical antifungal agent, and oral ampicillin and griseofulvin. Three weeks prior to presentation he had developed nocturnal fevers, palpitations and intermittent aching in the left side of the chest. Further systematic enquiry was negative.

His past medical history included idiopathic unilateral testicular atrophy and a retropubic prostatectomy for benign prostatic hypertrophy 8 years previously.

Examination

Physical examination revealed a well nourished, afebrile man with a flushed face. There were striking, annular, erythematous, purpuric plaques distributed on the anterior neck (Fig. 1), cheek, forehead and lumbar region (Fig. 2). Slight lymphadenopathy was present in the left side of the neck and left axilla only. There was no hepatomegaly, but the spleen was grossly enlarged to the level of the umbilicus. Examination of the oral cavity showed pharyngitis, and purpura on

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Case details were presented to the Clinico-pathological conference of the Section of Dermatology, Royal Society of Medicine, December 1986. Reproduced here with permission of the Editorial Representative, Journal of the Royal Society of Medicine.

Correspondence: Dr R.A. Logan.

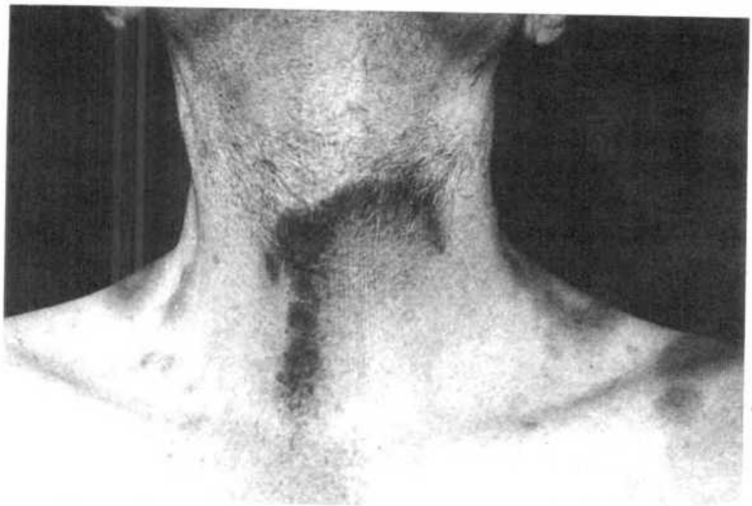

FIGURE 1. Annular purpuric plaques around the neck at presentation.

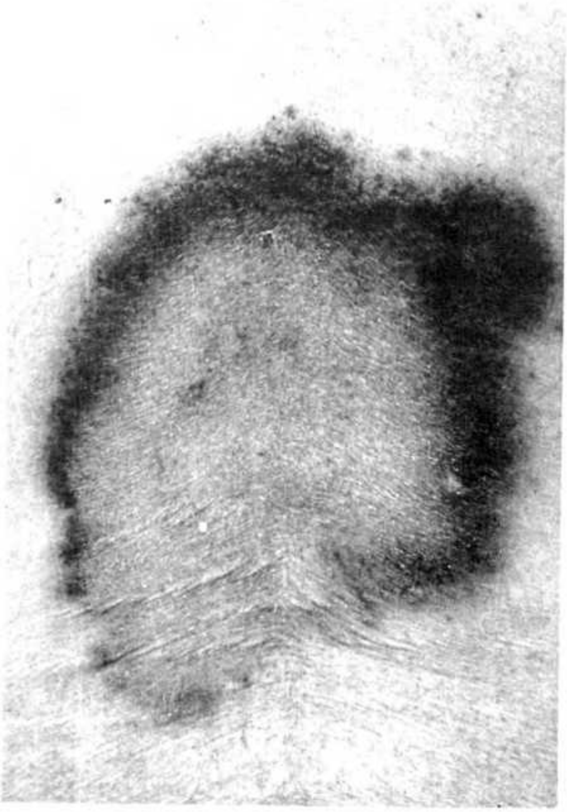

FIGURE 2. A 10 cm diameter purpuric plaque showing central clearing, present on the lumbar region.

the soft palate, but no gingival disease or oral ulceration. The rest of the physical examination was unremarkable.

Some of the more nodular skin lesions resembled those seen in Sweet's syndrome. Because of the known association of this syndrome and various types of myeloproliferative disorders[1] a blood count was performed immediately. This indicated a leukaemic process and the patient was immediately referred to the Haematology Department of the Middlesex Hospital for further assessment and management.

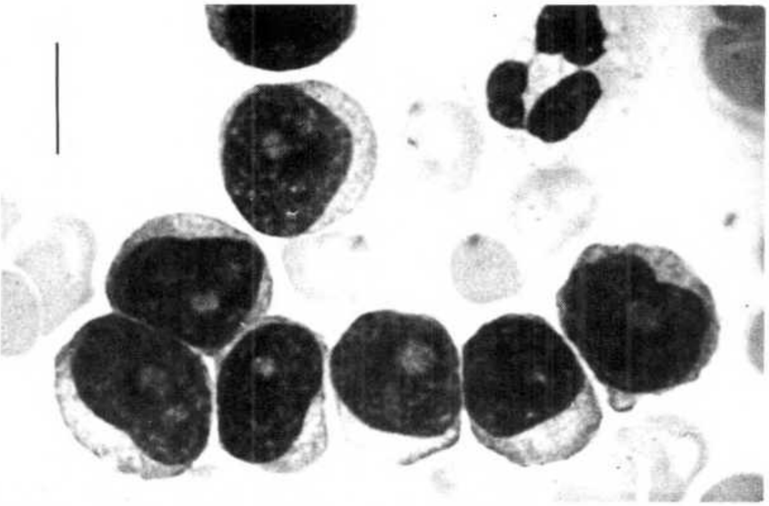

FIGURE 3. High power view of prolymphocytes in a peripheral blood film, showing prominent nucleoli and moderately abundant cytoplasm (Giemsa stain; original × 3000; Bar = 10μm).

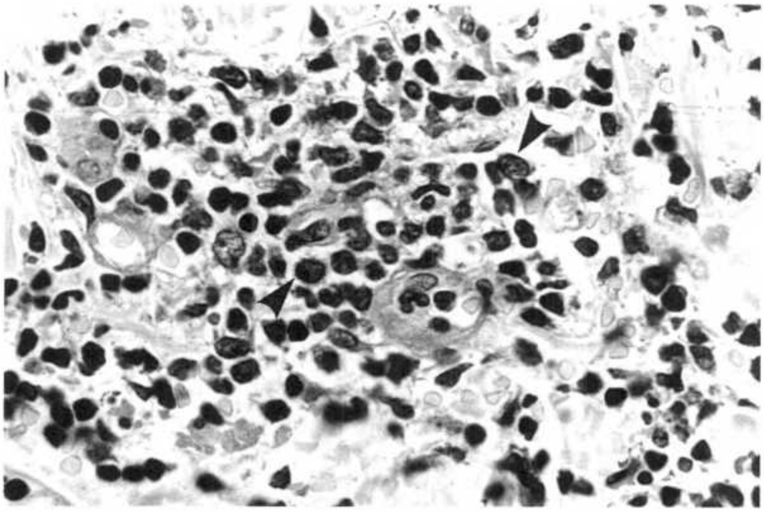

FIGURE 4. High power view of mononuclear cell infiltrate in the dermis. Many of the cells resemble prolymphocytes, having more cytoplasm than normal lymphocytes, and larger nuclei with prominent nucleoli (arrows). Red cell extravasation can also be seen. (Haematoxylin and eosin; original × 500).

Investigations

Laboratory investigations showed, Hb 16·1 g/dl, WBC 200 × 10^9/l, platelets 49 × 10^9/l and ESR 3 mm/h. The gross elevation of white cells was due almost entirely to prolymphocytes (Fig. 3). Marker studies showed these cells to have a CD4+ve phenotype (T-helper cells).

Histopathology of one of the plaques on the neck showed changes compatible with leukaemia cutis. A mononuclear cell infiltrate was present in the upper and mid-dermis with collagen dissection in 'Indian file' fashion. Under high power many of the infiltrating mononuclear cells had cytological features similar to those in the peripheral blood film, resembling large lymphocytes with prominent nucleoli (Fig. 4). They did not stain with chloracetate esterase. Lymphocyte marker studies (using UCHL1 for T cells and 4KB5 for B cells) showed that the infiltrate was composed of cells of T and B cell lineage in approximately equal numbers. Red cell extravasation was present without evidence of vasculitis.

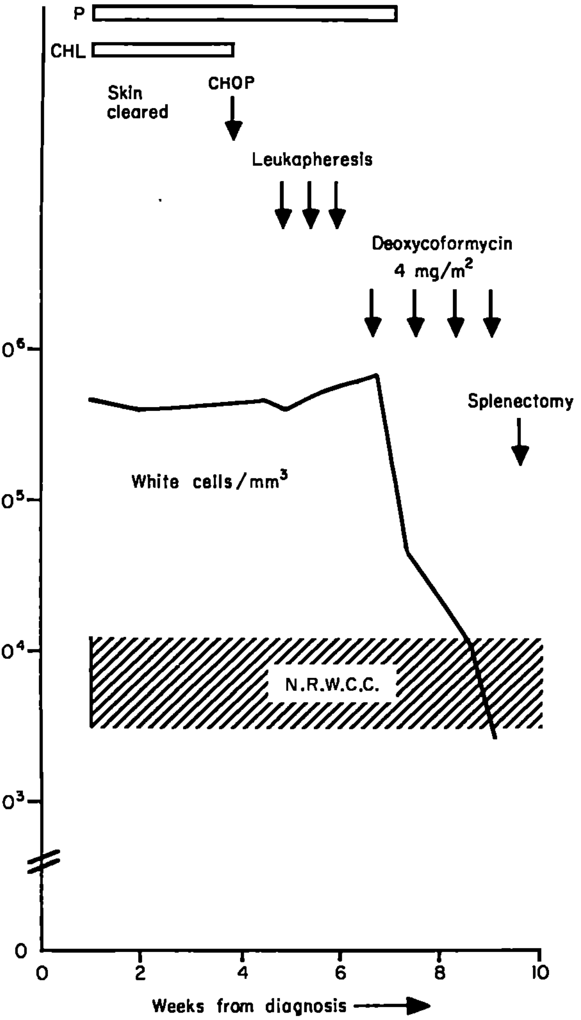

FIGURE 5. Effect of treatments on skin lesions and peripheral white cell count. Solid line = white cell count; NRWCC = normal range for white cell count; P = prednisolone; CHL = chlorambucil; CHOP- = cyclophosphamide, adriamycin, vincristine and prednisolone.

Course

A diagnosis of prolymphocytic leukaemia was made on the basis of the clinical presentation with gross splenomegaly, minimal lymphadenopathy and a markedly raised white cell count due to proliferation of prolymphocytes.

The patient's clinical progress and white cell count is shown diagrammatically in Figure 5. Three days after his initial presentation he had developed a swinging pyrexia and malaise. Treatment was begun with a combination of chlorambucil 10 mg/day and prednisolone 40 mg/day. Both the fever and the skin lesions settled within a week on this regimen.

However, over the next three weeks there was an inexorable rise in the leukocyte count, splenomegaly persisted, and he developed hyperviscosity, thrombocytopaenia and anaemia requiring blood transfusion. A course of combination chemotherapy (cyclophosphamide, adriamycin, vincristine and prednisolone), and leukapheresis on three occasions made no impression on the disease. He developed cardiac failure and increasing signs of hyperviscosity. After 6 weeks the leukocyte count had risen to 700×10^9/l.

At this stage he was commenced on deoxycoformycin 4 mg/m^2, four doses being given intravenously over the following 2 weeks, in conjunction with prophylactic oral cotrimoxazole and amphotericin B. This led to a dramatic fall in the peripheral white cell count (Fig. 5) and a reduction in splenic size. Splenectomy was then possible and was carried out uneventfully three weeks after the first dose of deoxycoformycin.

Over the ensuing 9 months the patient remained in good general health, with no recurrence of the original skin lesions. However, periodic elevations in the white cell count required three further courses of deoxycoformycin. After each course the patient noticed an exacerbation of erythema of the face, neck and conjunctivae with palpable papular areas on the face. This is considered to represent an exacerbation of pre-existing rosacea rather than a manifestation of the leukaemic state.

DISCUSSION

Prolymphocytic leukaemia is a rare and distinctive variant of chronic lymphocytic leukaemia (CLL), first described in 1974.[2] It characteristically presents in middle-aged males, with fatigue, weight loss, fevers and massive splenomegaly, but little or no lymphadenopathy. The peripheral white cell count is usually grossly elevated due to a proliferation of prolymphocytes. Unlike CLL, response to treatment with alkylating agents is poor with a median survival time of 6 months from diagnosis (Dr D. Catovsky, personal communication 1987).

In the original series of 15 cases only one patient had skin involvement at presentation, in the form of cutaneous purpura and nodules.[2] A further four patients developed purpura during the course of their disease and one other developed cutaneous nodules. In a recent series (Dr D. Catovsky, personal communication 1987) 10 out of 41 patients (24%) with prolymphocytic leukaemia had skin involvement. These included a widespread subcutaneous nodular eruption and a case with erythroderma. The case reported here is, therefore, unusual in that it was the cutaneous lesions, rather than the systemic symptoms of the leukaemic state that were the predominant presenting feature. However, these lesions cannot be considered specific for this disease as a wide variety of skin lesions may occur in all types of leukaemia. It is also recognized that skin lesions may present before the development of systemic symptoms from the leukaemic state.[3]

In the present case the skin lesions responded rapidly to a combination of chlorambucil and prednisolone. However, the leukaemic state remained refractory to conventional treatment.

Remission was achieved using deoxycoformycin, an experimental anti T cell agent. This drug has recently been reported to have induced remission in two further cases of prolymphocytic leukaemia of T helper phenotype.[4] Use of this drug in the present case allowed splenectomy to be carried out more safely. Splenectomy was first advocated in this condition by earlier workers who felt that the spleen may be a major site of production of the abnormal prolymphocytes.[2]

At the time of writing our patient was alive and well, 14 months after the onset of his symptoms.

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We wish to thank Dr David Linch and colleagues in the Department of Haematology, Middlesex Hospital, London for their expert management of this case. Dr Daniel Catovsky of the MRC Leukaemia Unit, Royal Postgraduate Medical School, London provided helpful information.

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Acute psoriasis associated with Lancefield Group C and Group G cutaneous streptococcal infections

CATRIONA A.HENDERSON AND A.S.HIGHET

Department of Dermatology, York District Hospital, Wigginton Road, York YO3 7HE, U.K.

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SUMMARY

Involvement of *Streptococcus pyogenes* (β haemolytic streptococcus, Lancefield Group A) infection, usually of the throat, in acute exacerbations of psoriasis is well known. We report here two cases of an acute psoriatic eruption associated in one patient with Group C streptococcal cellulitis and in another with Group G streptococcal intertrigo.

CASE REPORTS

Case 1

A 73-year-old lady was admitted for treatment of recurrent bilateral venous ulcers, and widespread psoriasis affecting mainly the trunk and flexures, of one week's duration (Fig 1). She had no previous or family history of psoriasis. Although she was afebrile, low grade cellulitis was suspected because of malaise, erythema and swelling of the lower legs. Light growths of a Lancefield Group C streptococcus, *Staphylococcus aureus* and *Pseudomonas aeruginosa* were obtained from the right leg ulcer. After 1 week on flucloxacillin 2 g/day and 2 weeks on emollients the psoriasis had cleared and 2 years later there had been no recurrence.

Case 2

A napkin eruption which developed in a baby girl at the age of 7 months appeared initially irritant, but was slow to improve, and at the age of 15 months psoriasiform features were noted, with scattered psoriatic lesions elsewhere. At 16 months she was seen with an acute flare-up of the napkin eruption with a purulent exudate in the groins, and with acute widespread guttate psoriasis. A moderate growth of a Lancefield Group G streptococcus and a heavy growth of *Staph. aureus* were obtained from a groin surface swab. After a week on oral erythromycin 2 g/day and Triadcortyl® ointment (triamcinolone, neomycin and nystatin) signs of infection had cleared and the psoriasis had improved. Over the subsequent 8 months she was treated with

Correspondence: Dr C. A. Henderson.

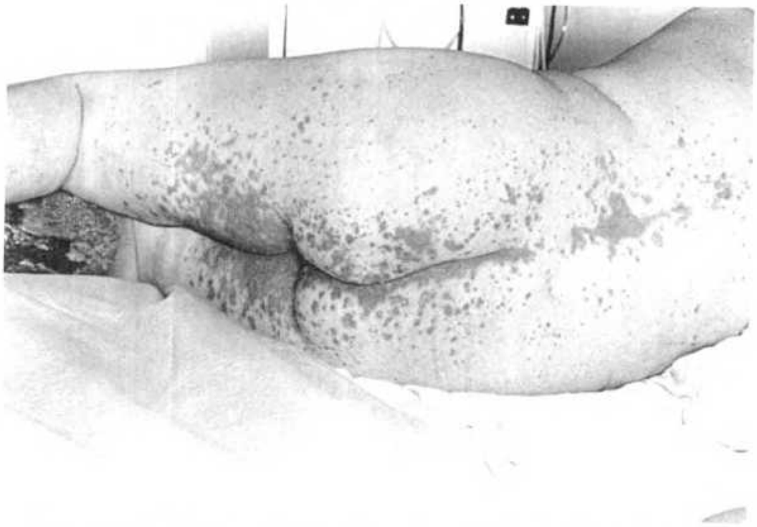

FIGURE 1. Back view of Case 1 showing distribution of psoriatic lesions.

Nystaform-HC® ointment (hydrocortisone, chlorhexidine and nystatin) applied to the napkin area, with satisfactory control and without psoriatic lesions elsewhere.

DISCUSSION

Our patients had acute eruptions of psoriasis associated with cutaneous streptococcal infection; cellulitis involving a Group C streptococcus (Case 1) and flexural psoriasis infected with a Group G streptococcus (Case 2). *Staph. aureus* was also present in each case, but that organism has not been implicated previously in psoriasis.

It is widely accepted that acute exacerbations of psoriasis, especially of the guttate type, may follow streptococcal infections after an interval of 1–3 weeks.[1-3] The mechanism of this effect is not established, but activation of the alternative complement pathway may be involved;[4] alterations in this pathway have been documented in psoriatic patients in general[5] and streptococcal structures (mainly membranes) can activate it by a properdin-independent mechanism.[6]

The site of the preceding streptococcal infection has nearly always been the throat, although one case following an episode of erysipelas was mentioned by Norrlind.[1] We have not come across reports implicating more superficial cutaneous streptococcal infections. Streptococcal isolates have nearly always belonged to Lancefield Group A;[2] we know of no reports incriminating other groups in the acute eruption of psoriasis. Elevation of ASO titre has been adduced to support the triggering role of streptococcal infection,[1,2] but it should be noted that raised levels may reflect recent infection with Groups C or G streptococci in addition to Group A. Tauber's complement studies[6] mainly involved Group A organisms, but similar effects were seen with strains belonging to Groups C, D and G.

Streptococcal impetigo is a major precursor of post-streptococcal acute glomerulonephritis;[7,8] in addition, post-streptococcal nephritis is not confined to Group A infections, as cases have followed infection with Group C organisms.[9] Thus, in principle, systemic sequelae may follow superficial streptococcal infection, and infection with non-Group A organisms.

A chance association between the streptococcal infections and the eruptions of psoriasis in our cases is possible. However, the extensive eruptions developed acutely, and settled rapidly with treatment which included appropriate antibiotics. Case 1 had no history of psoriasis before or since; Case 2 had a chronic psoriasiform napkin eruption, but the widespread psoriasis was at its most severe when the infective intertrigo was diagnosed.

These cases suggest that, in addition to the classical Group A streptococcal pharyngitis, streptococcal infection precipitating or aggravating psoriasis may be cutaneous and may involve Lancefield Groups C and G organisms.

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Recurrent solitary giant trichoepithelioma located in the perianal area; a case report

S. BECK AND D.W.K. COTTON*

Departments of Pathology, Rotherham District General Hospital, Moorgate Road, Rotherham and * University of Sheffield Medical School, Beech Hill Road, Sheffield S10 2RX, U.K.

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SUMMARY

A case of giant solitary trichoepithelioma of the perianal region is described. Following excision the lesion recurred with a similar histological appearance. This is the first report, to our knowledge, of recurrence of such a lesion.

Tatnall and Wilson Jones[1] described three cases of very large trichoepitheliomas occurring in the perianal region as solitary lesions. They reported the successful excision of these lesions and commented, in one case, that there was no recurrence. In the present report we describe a further case of solitary giant trichoepithelioma of the perianal region that was excised and which recurred 17 years later.

CASE REPORT

A 31-year-old man presented in 1969 with a small swelling on the left side of the scrotum. This was excised under general anaesthaesia. There were no other clinical features of note. Since the histology showed a tumour of low grade malignancy that was possibly incompletely excised, the patient was followed up at 6-monthly intervals in the out-patient clinic until 1976 when he was discharged. At no time during this period was there any evidence of recurrence or lymphadenopathy. He presented again in 1986 with a 5-month history of a nodule in the scar from the previous excision. On examination there was a 3cm nodule in the excision scar on the left side of the scrotum; there was no lymphadenopathy and no other clinical signs of note. The lesion was excised under general anaesthaesia. The patient was regularly followed up and had no signs of further recurrence after 1 year.

Histology

The first lesion was a 2cm nodule consisting of uniform basiloid cells forming small nodules and cystic spaces containing amorphous eosinophilic material. The clumps showed pronounced

Correspondence: Dr D.W.K. Cotton.

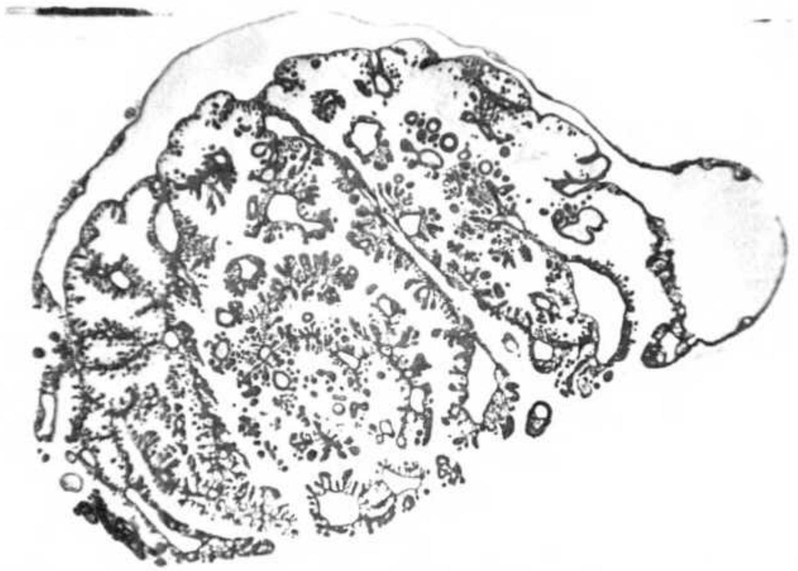

FIGURE 1. Low power view of the recurrent lesion showing the hair follicle arrangement. (Original × 6).

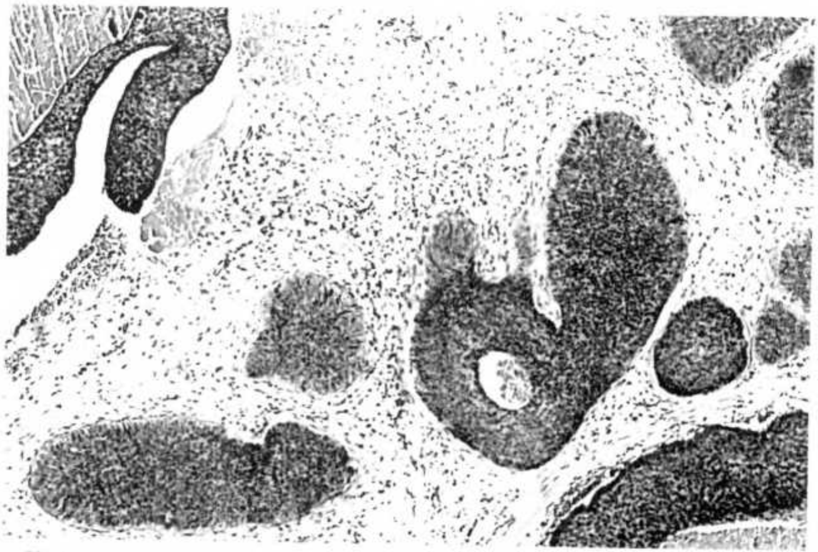

FIGURE 2. High power view of tumour clumps from the original lesion showing clefting at the stromal interface and the epitheliomatous arrangement. (Original × 60).

budding and the low power pattern, therefore, looked rather like a trichofolliculoma (Fig. 1). At high power it could be seen that this pattern was deceptive since there was no real organized differentiation and the whole lesion was epitheliomatous. Mitoses were frequent, but normal in form and they were limited mainly to the peripheral regions of the tumour nodules. The tumour clumps showed peripheral palisades and were surrounded by a marked basal lamina; a minor degree of clefting at the stromal interface was present (Fig. 2). The intervening stroma showed

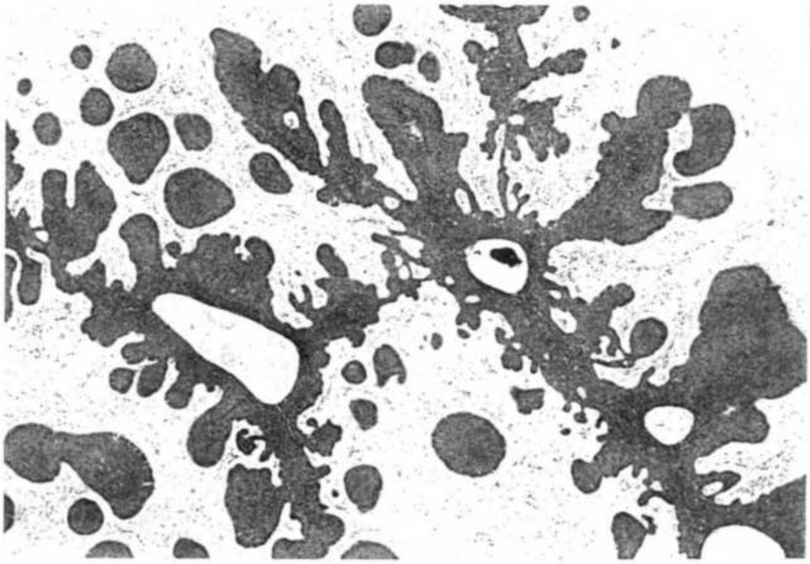

FIGURE 3. High power view of the recurrent lesion showing a small focus of calcification. (Original × 40).

some evidence of scarring and contained numerous mast cells. No amyloid substance was demonstrated. In general the stroma was cell rich and myxoid in appearance with numerous small vessels. The spindle cells of the stroma appeared to be arranged in laminae around the tumour clumps and there were very occasional foci of primitive hair papillae formation.

The recurrence was a 3cm nodule with an identical appearance to the first tumour, including occasional foci of calcification within the tumour nodules (Fig. 3).

DISCUSSION

The histological appearance of these two tumours is identical to that described by Tatnall and Wilson Jones.[1] These tumours seem to be epitheliomatous hair follicle tumours, but are distinct from basal cell carcinomas. Both solitary and multiple trichoeptheliomas are well known tumours, thought to be of hair follicle origin,[2] and solitary giant forms have also been described.[3] However, the occurrence of giant solitary forms in the perianal region has only been described, to our knowledge, in one previous report.[1] The previously reported cases were noted to extend deeply and to involve subcutaneous fat. Our patient's first lesion appeared to be completely excised, but no fat was taken with the excision and it is possible that there were nests of tumour remaining. The very long delay before the recurrence presented may indicate the slow growth of these lesions, but it also suggests that the second lesion could have been a second primary. The extreme rarity of the lesion, however, would seem to make this very unlikely. It does, however, seem prudent to follow up these patients over a very long period since it is now apparent that recurrence is a real risk.

ACKNOWLEDGMENTS

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Primary cutaneous adenoid cystic carcinoma: case report, immunohistochemistry, and review of the literature

TH.H VAN DER KWAST, V.D.VUZEVSKI, F.RAMAEKERS,†
M.T.BOUSEMA* AND TH. VAN JOOST*

Departments of Pathology and *Dermatology, Erasmus University, Rotterdam and †Department of Pathology, University Hospital of Nijmegen, The Netherlands

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SUMMARY

We report a case of primary cutaneous adenoid cystic carcinoma in a 75-year-old man.

Electron microscopy revealed similar features to those previously described in adenoid cystic carcinomas of salivary gland origin: wide intercellular spaces, pseudocysts containing replicated basement membrane-like material and true lumina lined by cytoplasmic membranes with numerous microvilli. Immunohistochemistry using antibodies to several cytoskeletal proteins (keratins and actin) indicated the presence of two epithelial tumour cell populations, one with the phenotype of myoepithelial cells, lining the pseudocysts and the periphery of the tumour fields, and another with the phenotype of acinar cells of the secretory coil of sweat glands. In addition, the tumour showed immunoreactivity for epithelial membrane antigen, but not carcinoembryonic antigen. A review of the literature on other cases of primary cutaneous adenoid cystic carcinoma showed that this tumour generally affects older patients with a female to male ratio of 4:1. The most common tumour site appears to be the scalp (40%), and the recurrence rate is 50%. Surgical treatment with extensive resection margins is recommended.

Adenoid cystic carcinomas (ACC) most commonly originate in the major and minor salivary glands. Other, rare, primary locations may be the breast,[1] the major bronchi,[2] the uterine cervix,[3] the external auditory canal,[4] and the skin.[5–16] Cutaneous localization of ACC may also result from direct extension of ACC of the minor salivary glands situated in the paranasal sinuses,[17] and rarely a distant cutaneous metastasis of a primary salivary gland ACC occurs.[18] Primary cutaneous ACCs are generally considered as eccrine sweat gland carcinoma.[19] Electron microscopic and immunofluorescence studies have implicated myoepithelial cells as the histogenetic precursors of ACC of the salivary glands and uterine cervix.[20,21]

Correspondence: Th.H van der Kwast, M.D., Ph.D., Department of Pathology, Erasmus University Rotterdam, P.O. Box 1738, 3000 DR Rotterdam, The Netherlands.

The expression of a distinct set of keratins and other cytoskeletal proteins (e.g. actin) by tumour cells may permit a more detailed characterization of the various epithelial cells within a given tumour.[22] We present here histology, electron microscopy and immunohistochemistry of a case of primary cutaneous ACC, together with a review of the literature.

CASE REPORT

A 75-year-old Caucasian man presented with a tender, mobile tumour, 2 cm in diameter, on his chest. The nodule was firm, reddish in colour, with an irregular lobular surface and had slowly increased in size over several years. The lesion was surgically excised and histopathology revealed an adenoid cystic carcinoma. The resection margins were not free of tumour. Nine months later a firm tumour 2 cm in diameter recurred in the scarred area. The lesion was removed surgically and the histopathological diagnosis of adenoid cystic carcinoma was again established. No pathology of the salivary glands or the external auditory canals was found.

METHODS

Tissue

All tissue obtained at the first excision was fixed in 10% phosphate buffered formalin and processed routinely for microscopic examination. Part of the recurrent tumour was also processed for electron microscopy and a few slices of tumourous tissue were snap frozen in chilled isopentane and stored in liquid nitrogen.

For electron microscopy, tissue samples obtained immediately after surgical removal of the tumour were cut into small cubes, fixed in a solution of glutaraldehyde-formaldehyde, postfixed in phosphate buffered osmium-tetroxide, dehydrated in a graded series of ethanols, and embedded in Epon resin. Semithin 1 μm sections stained with toluidin-blue permitted the

TABLE 1. Antibodies used in the study

Antibody	Antigen recognized	Antibody type	Source
RCK 105	keratin 7	mouse monoclonal	Dr F. Ramaekers[23]
RKSE 60	keratin 10	mouse monoclonal	Organon Technika, Holland[24]
RGE 53	keratin 18	mouse monoclonal	Organon Technika, Holland[25]
ER-TR5	keratin (undefined)	rat monoclonal	Dr E. van Vliet[26]
pKer	several keratins	rabbit polyclonal	Organon Technika, Holland[27]
pVim	vimentin	rabbit polyclonal	Sanbio, Holland[28]
RAc1	actin	mouse monoclonal	Dr F. Ramaekers
12E9	epithelial membrane antigen	mouse monoclonal	DAKO, Denmark[29]
PARLAM-4	carcinoembryonic antigen	mouse monoclonal	Organon Technika, Holland

selection of cribriform pseudocyst containing areas for thin sectioning. Ultrathin sections were stained with uranyl acetate and examined in a Zeiss EM 902 electron microscope.

Antibodies

The antibodies used in the study are listed in Table 1. The monoclonal antibodies directed against epithelial membrane antigen (EMA), carcinoembryonic antigen (CEA), and the polyclonal antibodies directed against vimentin and keratin were applied to deparaffinized tissue sections. For demonstration of keratin and vimentin, deparaffinized tissue sections were pre-treated with 1% pronase (Sigma) for 25 min at 37°C. The monoclonal antibodies specific for actin (RAc1), keratins 7, 10 and 18 (RCK 105, RKSE 60 and RGE 53, respectively) and the rat monoclonal antibody ER-TR5 were only applied to frozen sections, since no immunostaining on formalin-fixed or paraffin-embedded sections has been achieved.[23] ER-TR5 is a rat monoclonal antibody specific for a restricted number of mouse and human epithelia. Immunoelectron microscopy has shown that it specifically stains intermediate filaments of epithelial cells.[26] The immunoperoxidase technique on paraffin-embedded and frozen tissues was performed using the indirect conjugated peroxidase method as described elsewhere.[26] In order to visualize the binding of the IgM monoclonal anti-actin antibody, an FITC-conjugated goat anti-mouse IgM(Fc) was used.

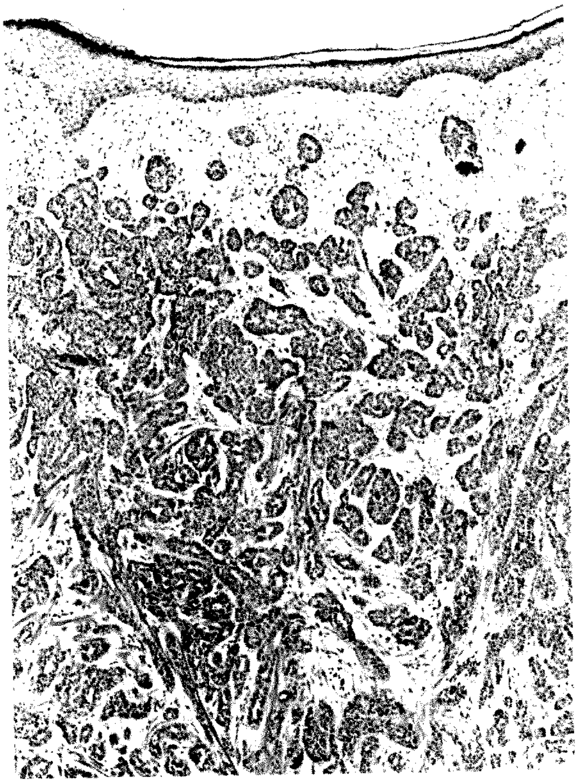

FIGURE 1. Cribriform and tubular tumour masses of ACC localized in the reticular dermis and extending into the subcutis. No contiguity with the epidermis. (Haematoxylin and azophloxin stain; original × 125).

RESULTS

Histopathology and electron microscopy

The primary and recurrent tumours were composed of tubular and cribriform epithelial cell masses distributed throughout the reticular dermis and extending into the subcutis. The papillary dermis was not involved. No connection with the epidermis or hair follicles was observed (Fig. 1). The tumour cells had a basaloid appearance, and contained uniform round to oval somewhat vesicular nuclei with an occasional rather conspicuous solitary nucleolus. The cytoplasm was sparse and several mitoses were present. Palisading of tumour cells was not evident. Most of the lumina of the tubular and cribriform masses were empty, but some contained homogeneous material that reacted with periodic acid-Schiff, periodic acid-Schiff-diastase, and alcain blue (pH 2·5) stains. The latter staining reaction was abolished by pretreatment with hyaluronidase. In the primary and the recurrent tumour perineural invasion was found. Some tumour masses were surrounded by alcian blue positive material.

Electron microscopy of the tumour masses revealed wide intercellular spaces, while the cytoplasmic processes of the tumour cells were joined by desmosomes. The larger cystic spaces in the cribriform masses contained filamentous material (Fig. 2). True lumina were identified by the presence of junctional complexes between the surrounding cells and by the presence of numerous microvilli protruding into the lumen. The lumina contained flocculated material, most probably inspissated mucin. Two types of tumour cells could be distinguished: an electron-dense cell type with deeply indented nuclei, lining the pseudocysts (Fig. 3) and a less electron-dense cell type with round nuclei (Fig. 4). Both cell types contained numerous mitochondria and ribosomes. In some cells secretory vacuoles and Golgi membranes were observed.

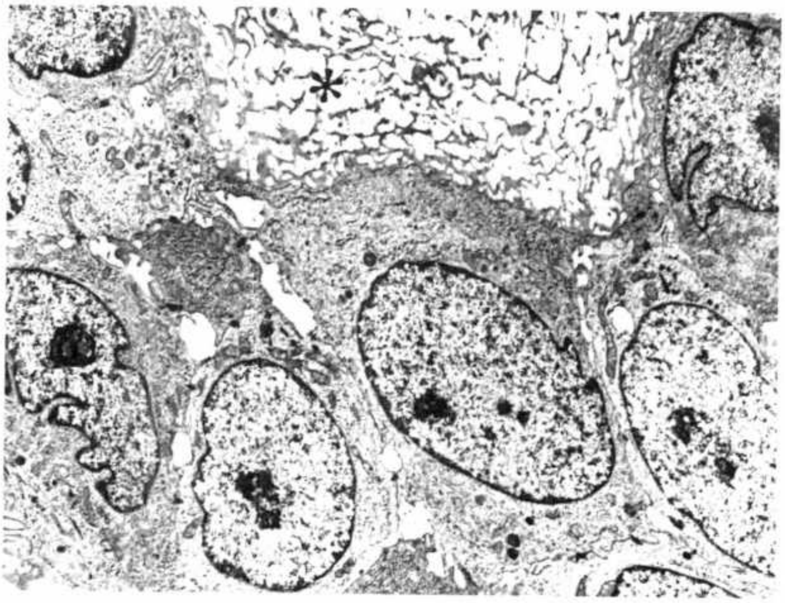

FIGURE 2. Electron micrograph of ACC. The pseudocysts contain filamentous material (asterisk). (Original × 3000).

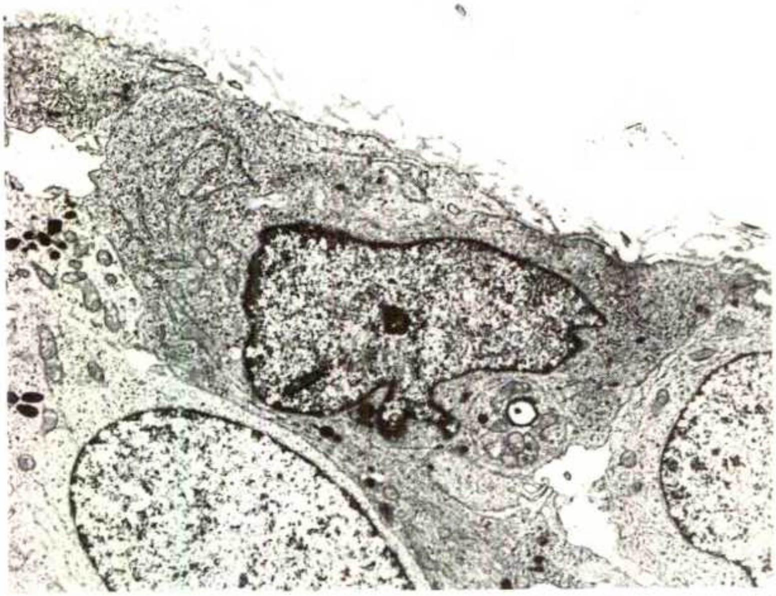

FIGURE 3. Electron micrograph of a myoepithelial cell. Note the elongated appearance of the cell, the electron-dense cytoplasm and the indented nucleus. (Original × 4400).

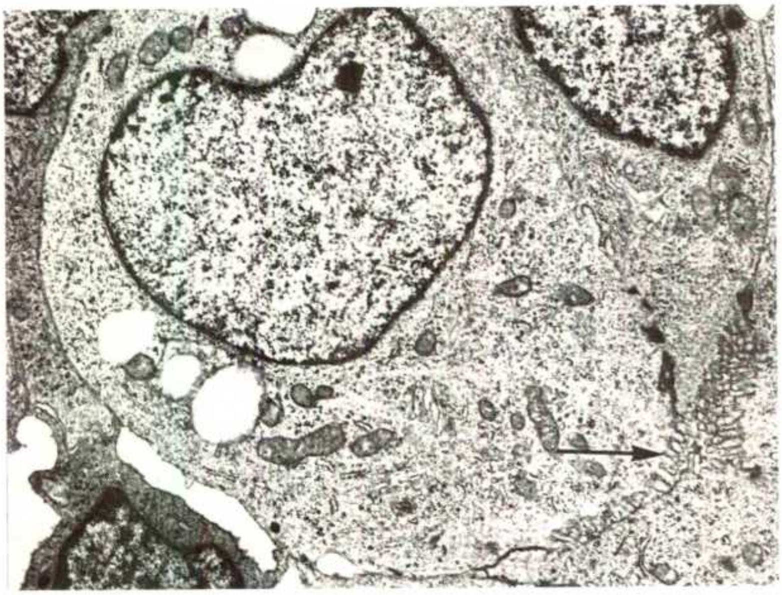

FIGURE 4. Electron micrograph of acinar cells showing abundant cytoplasm and numerous villous cytoplasmic projections (arrow). (Original × 4400).

Immunohistochemistry

The paraffin-embedded tumour material was immunoreactive for EMA and keratin, but no staining of tumour cells was found with antibodies to CEA and vimentin. EMA-positive material was found predominantly in the microcystic spaces of the tubular and cribriform tumour fields as well as on the surfaces of the cells lining the lumina of the tubular tumour strands. Faint cytoplasmic staining for EMA was present in a proportion of the cells at the periphery of the tumour fields. The larger pseudocystic spaces within the cribriform masses were not stained for EMA (Fig. 5). The polyclonal anti-keratin antiserum stained virtually all tumour cells, but a more intense staining was observed in the tumour cells lining the pseudocysts within the cribriform masses and in cells at the periphery of tubular and cribriform tumour masses. The monoclonal antibodies specific for keratins 7 and 18 gave intense staining of all epithelial tumour cells, except those lining the pseudocysts of the cribriform tumour fields and those at the periphery of the tubular and cribriform tumour fields (Fig. 6). No reactivity of tumour cells was observed with the monoclonal antibody specific for keratin 10. The rat monoclonal antibody ER-TR5 revealed a picture complementary to that observed with the antibodies against keratins 7 and 18. Thus, epithelial cells lining pseudocysts and those situated at the periphery of the tubular and cribriform tumour fields were moderately stained by ER-TR5. Occasional intensely ER-TR5 positive cells were, however, also present within the tumour fields.

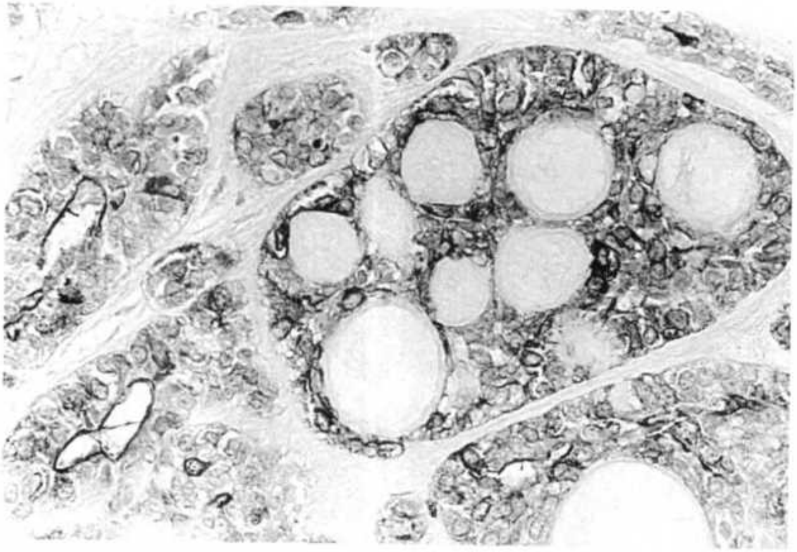

FIGURE 5. Cribriform and tubular tumor masses of ACC stained for EMA. The contents of the pseudocysts are not stained, while the lumina of the ducts and microcystic spaces are intensely EMA-positive. Nuclei counterstained with haematoxylin. (Original × 375).

Immunofluorescence studies on the expression of actin revealed an intense fluorescence along the circumference of the tumour fields and at the margins of the pseudocysts of the cribriform tumour masses (Fig. 7). A faint cytoplasmic staining was observed in the other tumour cells. Also occasional strongly actin positive tumour cells were present within the tumour fields.

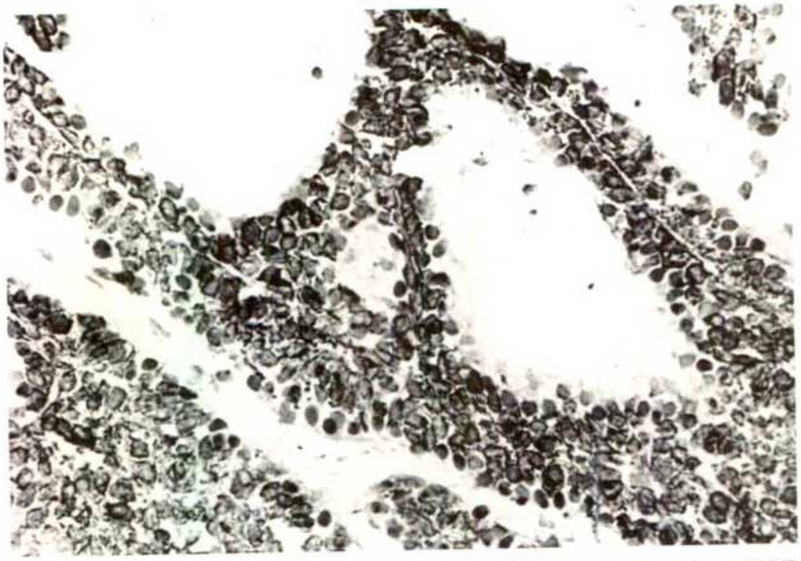

FIGURE 6. Cribriform masses of ACC stained for keratin 18 with monoclonal antibody RGE 53. The inner cells of the tumor show cytoplasmic staining. Cells lining the pseudocysts and at the periphery of the tumour masses are not stained. Nuclei counterstained with haematoxylin. (Original × 375).

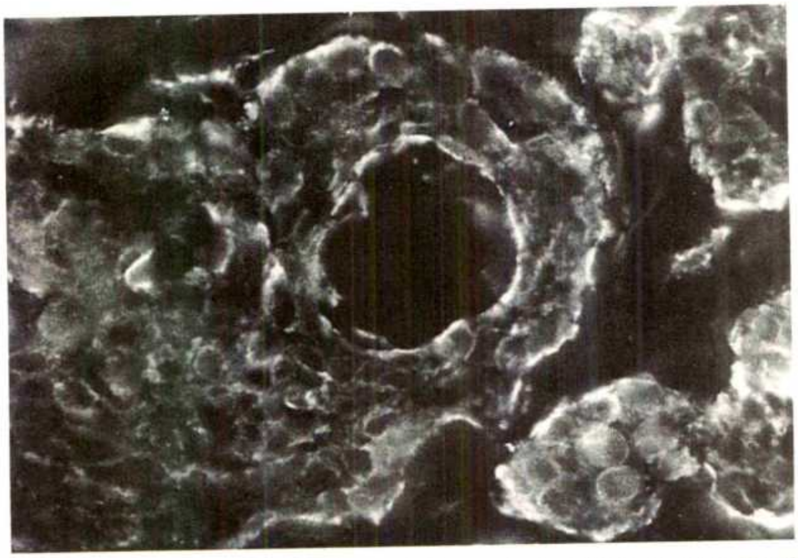

FIGURE 7. Cribriform masses of ACC stained for actin. Intense immunofluorescence staining of the outer rim of the tumour masses and along the pseudocysts can be seen. The remaining tumour cells are faintly stained. (Original × 250).

DISCUSSION

The cutaneous tumour described here fully meets the histopathological criteria for the diagnosis of an ACC. The ultrastructural features, including the presence of basement membrane-like material within the so-called pseudocysts,[30] further confirm the diagnosis of ACC. The differential diagnosis of cutaneous ACC must include adenoid type basal cell carcinoma.[19] Adenoid type basal cell carcinoma may also show a cribriform growth pattern and areas of cystic degeneration. The presence of palisading in solid tumour masses and contiguity of the tumour with overlying epidermis or hair follicles are the main histopathological criteria by which adenoid type basal cell carcinoma can be distinguished from ACC. In addition, from the results of Wick and Swanson[15] and the present findings it is apparent that immunohistochemistry can contribute to the separation of adenoid type basal cell carcinoma and cutaneous ACC. Cutaneous ACCs express EMA, in contrast to adenoid type basal cell carcinomas which do not. However, the presence of CEA in cutaneous ACC reported by Wick and Swanson[15] was not confirmed in the present study, using a monoclonal anti-CEA antibody. Probably this discrepancy is due to a difference in epitope specificity of the monoclonal antibodies used.

Our results on frozen sections indicate that cutaneous ACCs consist of two populations of epithelial cells. One population is made up of keratin 7-positive, keratin 18-positive cells, which express small amounts of actin. This population of tumour cells has a well circumscribed localization on the inside of the tumour fields and is surrounded by the population of epithelial tumour cells not expressing keratin 7 or 18, but high levels of actin. In addition, this population of cells possesses intermediate filaments recognized by ER-TR5. No immunoreactivity of any of the tumour cells was found with antibodies to keratin 10 or vimentin.

The apparent lack of detectable expression of vimentin in the present case of cutaneous ACC contrasts with the previously reported vimentin-positivity of six ACC of salivary gland origin.[31] The absence of vimentin in the myoepithelial cells of sweat glands[28] and the reported presence of this intermediate filament protein in triangular duct cells of the normal salivary glands[32] is in keeping with the contrasting observations in ACCs of salivary gland and sweat gland origin.

Keratins 7 and 18 are found to occur normally in glandular epithelia, but not in squamous epithelia such as the epidermis.[23,25] Keratin 18 is expressed by nearly all glandular epithelia, while keratin 7 has a more restricted pattern of expression. In the skin, keratins 7 and 18 are solely present in the acinar epithelial cells of the secretory coil of sweat glands.[23] Ductal epithelium and myoepithelial cells of sweat glands do not express keratins 7 or 18, nor do sebaceous glands or hair follicles. In the skin, keratins 7 and 18 must thus be considered highly specific for sweat gland acinar epithelial cells. In contrast, expression of keratin 10 is entirely restricted to epidermal epithelial cells.[24] Therefore, we consider the presence of keratin 7 and 18 and the absence of keratin 10 in cutaneous ACC as a strong argument for the sweat gland origin of this tumour type.

The polyclonal antikeratin antiserum used in the present study stains all epithelial cells of the epidermal appendages,[27] including the myoepithelial cells of the sweat glands (data not shown). Similarly, this antiserum was reactive with all epithelial tumour cells of cutaneous ACC. Moreover, a higher intensity of immunostaining was observed in the population of epithelial cells at the periphery of the tumour fields and those lining the pseudocysts in the cribriform areas. In the skin, ER-TR5 exclusively stains the basal cells of the epidermis and the myoepithelial cells of the sweat glands (data not shown). The presence of ER-TR5 in the population of epithelial cells lining the tumour fields and the pseudocysts is therefore highly suggestive of a myoepithelial origin. The intense staining of this cell population by our anti-

TABLE 2. Reported cases of cutaneous adenoid cystic carcinoma

Reference	Age (years)	Sex	Tumour location	Tumour size (cm)	Perineural invasion	Recurrence
Stout and Cooley[5]	52	F	abdomen	1	+	– (82 months)*
Miller[6]	66	F	arm	0·5	not reported	– (19 months)
Freeman and Winkelmann[7]	34	F	scalp	3	not reported	+ (40 years)
Boggio[9]	66	F	scalp	8	+	not reported
Sanderson and Batten[11]	46	F	scalp	5		+ (11 years) (including pulmonary metastasis)
Headington *et al.*[8]	60	F	scalp	not reported	+	+ (21 months)
Headington *et al.*[8]	41	F	axilla	2·5	+	– (18 months)
Dissanayake and Salm[10]	47	M	scalp	3	not reported	– (30 months)
Cooper, Adelson and Holthaus[12]	61	F	arm	8	+	+ (30 months)
Boggio[13]	59	F	suprapubic	7	+	+ (5 years)
Beck[14]	41	F	back	not reported	not reported	+ (35 years)
Beck[14]	40	M	hand	not reported	not reported	– (9 months)
Wick and Swanson[15]	76	F	scalp	2	–	– (10 years)
Lang, Metcalf and Maize[16]	63	F	back	not reported	–	+ (5 years)
Present report	75	M	chest	2	not reported	+ (9 months)

* Period of follow-up.

actin monoclonal antibody further substantiates this suggestion. Previous studies on salivary gland ACC[20] and uterine cervix ACC[21] also indirectly indicated the presence of myoepithelial cells in these tumours. The accumulation of basement membrane material in the pseudocysts of ACC may thus be the result of a stimulatory effect of neoplastic transformation of the myoepithelial cells which normally produce basement membranes.[33] The data from the present study did not enable us to establish whether the histogenetic precursor cell of ACC resides in the myoepithelial or the acinar cell population, or, more probably, in a precursor common to both these cell types.[34]

Cutaneous ACC is an uncommon tumour of the skin, which was first described as a separate entity in 1975 by Boggio[9] and Sanderson and Batten.[11] The review by Cooper *et al.*[12] included eight cases of cutaneous ACC. Since then five more cases have been reported in the literature. A summary of published cases is given in Table 2. To our knowledge, the case reported by Sanderson and Batten,[11] represents the only case of cutaneous ACC with a distant metastasis. All other cases of cutaneous ACC have been characterized by a prolonged indolent disease course, without distant metastases. ACCs in locations other than skin and breast seem to have a more aggressive disease course.[3,4] The recurrence rate of cutaneous ACC is 50% (Table 2). Recurrence seems not to be related to the presence of perineural invasion or to tumour size. The data show a female predominance of 4:1, and 40% of the tumours appear to originate in the scalp. Since irradiation of the tumour does not prevent recurrence,[14] surgical treatment with extensive resection margins is recommended.

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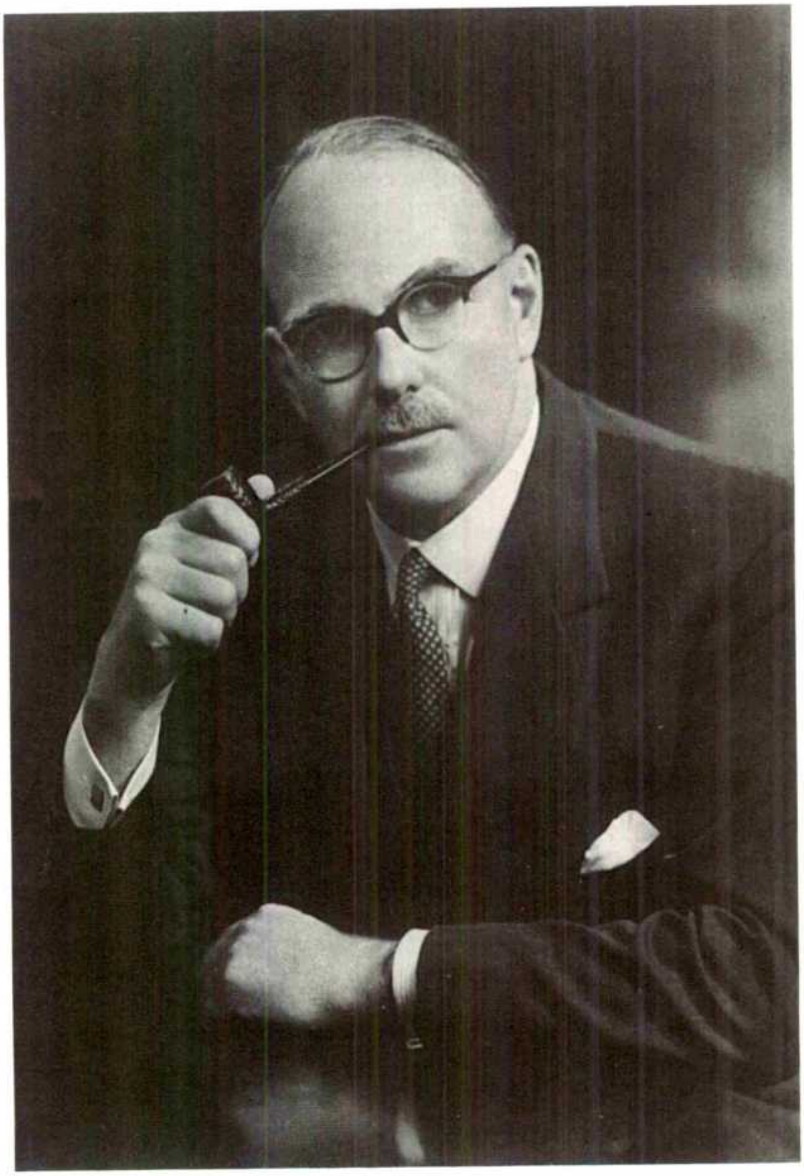
British Journal of Dermatology (1988) **118,** 579–580

Obituary

Dr G.A.Grant Peterkin
M.B.E., M.B.Ch.B., F.R.C.P.(Ed)

George A. Grant Peterkin, formerly consultant dermatologist at the Royal Infirmary, Edinburgh, and associated hospitals, died on 26th July 1987 aged 80 years.

He was a true Scot and proud of it. He was born in Forfar, Angus, the son of a general practitioner. He was educated at Forfar Academy, Merchiston Castle School and the University of Edinburgh where he graduated M.B.Ch.B. in 1929. In his final undergraduate year he was confined to hospital after a climbing



accident on Lochnagar. The severe and generalized rash which he developed after a course of tetanus antiserum was a significant factor in his decision to specialize in dermatology. Soon after qualification he was awarded a Leckie-Mactier scholarship to study dermatology in Copenhagen. In 1933 he was appointed assistant physician to the skin department, The Royal Infirmary, Edinburgh and later became a consultant dermatologist to Leith, Deaconess and Bangour Hospitals.

He joined the Army Medical Corps in 1942. After setting up a 200 bed skin hospital at Penshurst, Kent, he was posted in 1943 to Algeria with the 1st Army. As it progressed through Italy he took charge of 1000 beds for patients with skin diseases in Naples. For his work during the war he was appointed M.B.E. and awarded the American Medal of Freedom with Bronze Palm. The war was certainly a formative influence on him and it was said that he came back a mature man, having left something of a boy. During the war he also established close links and friendships with many young American dermatologists and after the war, when they became leaders of American Dermatology, he made a number of lecture tours to the United States of America. Many of his pupils were able to take advantage of this connection and travelled all over the United States.

After the war he resumed work in Edinburgh, becoming physician with joint administrative charge of the Skin Department at the Royal Infirmary in 1954 and also running a successful private practice. He retired from hospital practice in 1971. He was an international honorary member of the American Dermatological Association and an honorary foundation member of the Australasian Dermatological Association. He was a member of the British Association of Dermatologists from 1935 and its president in 1971. At various times he was also president of the Medico-Chirurgical Society of Edinburgh and served on the board of management of the Royal Infirmary, the national panel of consultants and the Medical Appeals Tribunal.

He was first and foremost a clinician and was most at home with his patients. His memory was remarkable and he was always pleased to diagnose unusual syndromes. He liked to follow up new lines of treatment and always had some different therapeutic suggestion up his sleeve when patients seemed desperate. They thought the world of him and appreciated his optimistic approach; many still ask after him in a way which hints that they are now having to live with the second best.

'Pete' enjoyed teaching, he made even commonplace conditions seem interesting and his teaching sessions were often memorable and occasionally theatrical. He coped with large clinics and groups of undergraduates and postgraduates with admirable ease. Patients, even those whose loquacity had to be cut short by a timely examination of the tongue and oral mucosa, and students alike, were impressed that they had seen an important man and had got, so to speak, their money's worth. Peter was an avid reader and had a store of apt quotations for most occasions. He tended not to resort to novel scientific ideas to answer questions from students because he knew that he would dig his own grave by doing so. Instead he relied heavily on his wealth of clinical experience, which he would describe graphically. Dermatologists from many parts of the world, particularly India, Pakistan and Australia still remember these teaching sessions vividly, and Pete with great affection.

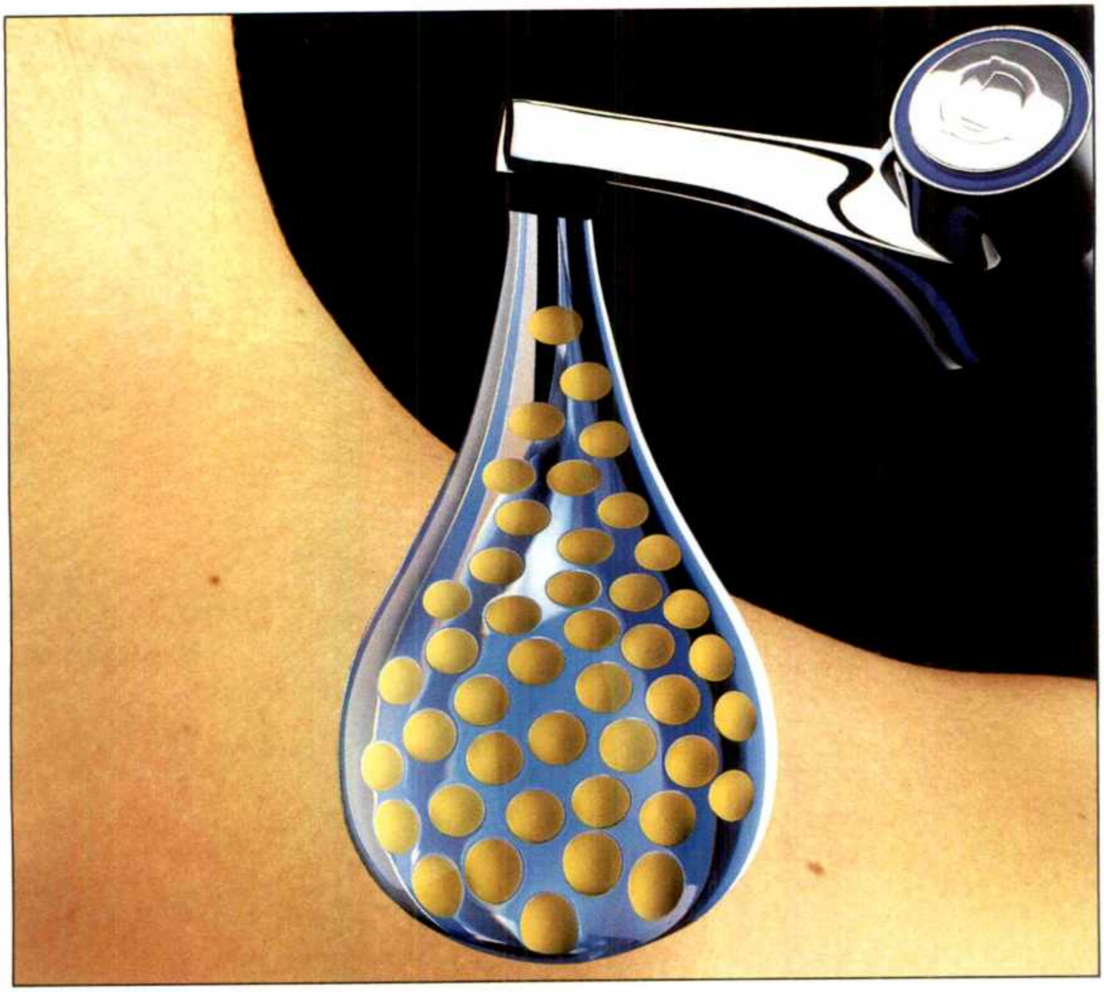
Dr Peterkin did not consider himself a research worker and never pretended to be one. However, he found time to write many papers describing his clinical observations, these have stood the test of time. In 1937 he was the first to report infection of human skin with the orf virus, and during the Second World War he recorded his experiences of many patients with conditions as diverse as impetigo, khaki dermatitis and eruptions due to sulphonamides and mepacrine. In 1949 he co-edited 'Common Diseases of the Skin' with the late R.Cranston Low.

Pete had many interests outside medicine. In his youth he had enjoyed rugby and hill climbing, but, as he grew older, his main sport was golf. He liked nothing better than battling it out with friends at Muirfield, where he was a most popular member of the Honourable Company of Edinburgh Golfers. He was an elder of the Kirk, a keen antiquarian and wrote a book on Scottish dovecots.

Grant Peterkin was devoted to his family. His first wife, Alison (Sally) died in 1974. He is survived by his second wife Sheena, two daughters and a son who, like his paternal grandfather, is a general practitioner in Forfar.

Nobody who had the good fortune of being his friend, colleague, pupil or patient will ever forget this big, kind and generous man.

J.A.A.Hunter



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British Journal of Dermatology (1988) **118,** 581–597.

Correspondence

A disposable tip for the Birtcher Hyfrecator

MADAM, The Birtcher Hyfrecator is a valuable tool for the electrocoagulation of small skin tags, spider naevi and small bleeding vessels during minor surgical procedures.

Unlike cautery, where the electrode tip may be sterilized by heating to red heat, the tip of the Hyfrecator remains relatively cool and thus should be separately sterilized by autoclaving after use.

With concern over the sterility of minor procedures, and the risk of transmission of blood borne infections, I describe an adaptor to allow the use of disposable needles as electrode tips on the Birtcher Hyfrecator.

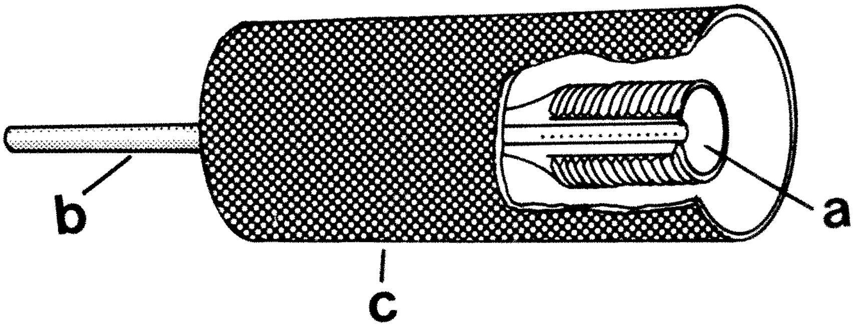

FIGURE 1. The adaptor. The dental needle screws into one end (a), the other end (b) plugs into the Hyfrecator handle. The nylon sheath (c) protects the user from accidental contact with the hub of the needle.

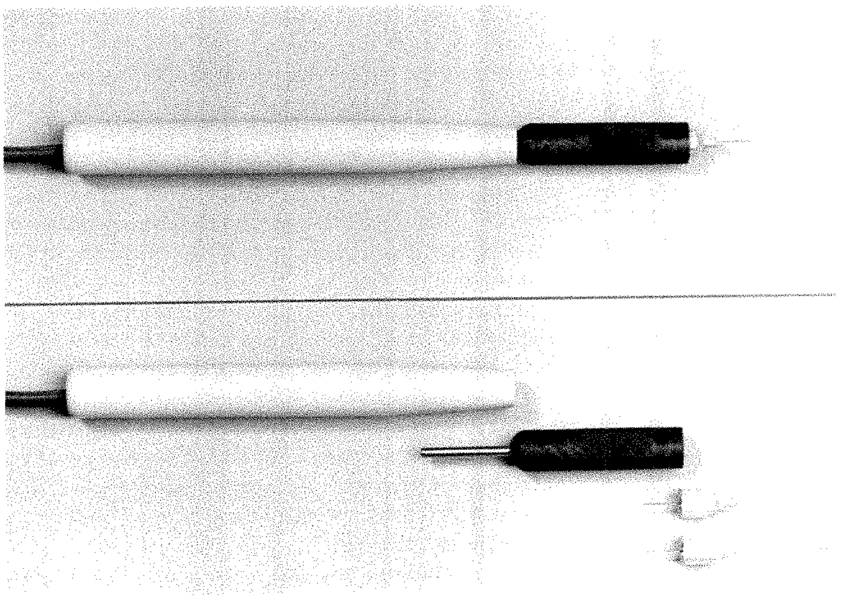

FIGURE 2. The adaptor is shown fitted to the standard Hyfrecator handle (top), and the component parts are shown separately (bottom).

The adaptor (Fig. 1) is constructed from a stainless steel core which has a screw thread and a hollow centre at one end (a), and a solid end to plug into the Hyfrecator handle at the other end (b). The core is insulated with a nylon sheath (c) which extends distally to cover the hub of the needle, protecting the user from accidental contact with it. Standard fitting metal hub dental needles are screwed into the end of the adaptor (Fig. 2).

I have used this adaptor with 27 and 30 g dental needles. In addition to providing a readily changeable sterile electrode, the small needle tip has the advantage that it can be used on even the smallest lesions and can be inserted just within a lesion or small vessel providing a more localized action. I have used this technique with success on both spider naevi and skin tags.

One important aspect of using the smaller tips is that the current density is higher than normal. When using the high output channel of the Hyfrecator a lower setting should be selected initially.

Department of Dermatology,
University Hospital of Wales,
Heath Park, Cardiff,
Wales, U.K.

R.J. MOTLEY

ACKNOWLEDGMENT

I am grateful to Mr H. Gay, Bioengineering Department, University Hospital of Wales, for constructing the adaptor.

Flow cytometric comparison of keratoacanthoma and squamous cell carcinoma

MADAM, A recent flow cytometric study (*British Journal of Dermatology* 1987; 117: 169) analysed the significance of DNA aneuploidy in keratoacanthoma and squamous cell carcinoma, amongst other cutaneous lesions. It was concluded that the two lesions could not be distinguished on the basis of DNA aneuploidy. Our recent preliminary studies of nuclear suspensions prepared, according to the method of Hedley,[1] from 31 cases of squamous cell carcinoma and 22 cases of keratoacanthoma are in agreement with this.[2] However, a number of limitations have emerged which need to be taken into account in the planning of future flow cytometric analyses in this area of continuing interest.

Firstly, the flow cytometric indices vary between differing degrees of differentiation of squamous cell carcinoma. Thus, while well and moderately differentiated squamous cell carcinomas showed DNA ploidy and proliferation indices (percentage of cells in G_2 and S phase of the cell cycle) which were not significantly different from those of keratoacanthomas (Table 1), those for the poorly differentiated group of squamous carcinomas were significantly different ($P < 0{\cdot}05$). In practice, however, it is only in the case of well differentiated squamous cell carcinomas, with significant mimicry of keratoacanthomas, that any putative diagnostic value of the flow cytometer may arise, since the more poorly differentiated squamous cell carcinomas do not cause diagnostic confusion.

The second caveat is that the age of a keratoacanthoma, as determined from the clinical history, may affect the cell proliferation cycle indices of this naturally-involuting lesion. We found a trend towards falling proliferation index with increasing age of the lesion (Fig. 1) in those cases where clinical duration data were available.

Finally, it has been shown that DNA ploidy and proliferation indices may vary between the peripheries and centres of various epidermal tumours,[3] although our study did not address this problem specifically. Thus, disparate results may be obtained depending on the area of the tumour sampled. The histological study of near-facsimile sections cut before and after the thick sections disaggregated for flow cytometry will at least allow verification of the area of tissue being analysed.

The similarities and differences between keratoacanthoma and squamous cell carinoma continue to provide fascination and we hope that these comments may be helpful to those wishing to use the resources of the flow cytometer in this area.

TABLE 1. Flow cytometric analysis of keratoancanthomas and squamous cell carcinomas (SCC)

	Number of cases	Mean G_1	Mean G_2	Mean proliferation index
Keratoacanthoma	22	82·2	7·5	14·1
Well differentiated SCC	15	82·5	10·4	11·7
Moderately differentiated SCC	12	83·8	7·2	12·6
Poorly differentiated SCC	4	88·2	5·4	9·5

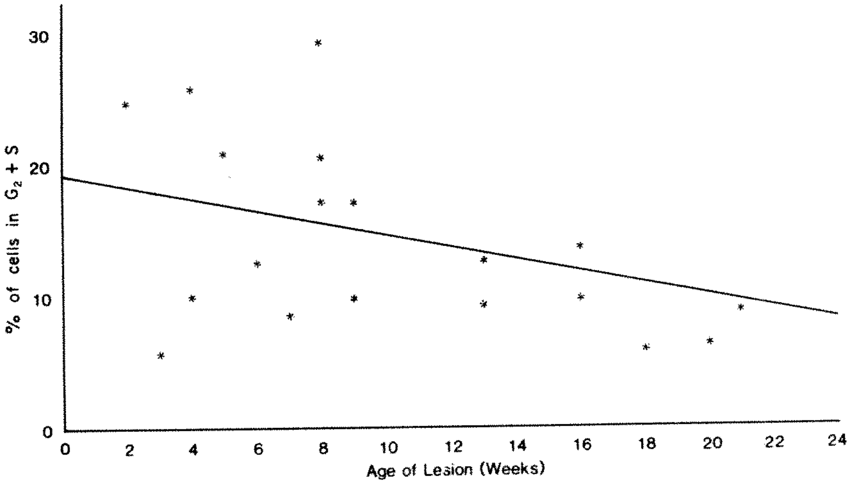

FIGURE 1. The relationship between mean proliferation index $(G_2 + S)$ and clinically-determined age of keratoacanthomas; $r = -0{\cdot}402$, $P > 0{\cdot}1$ $(y = 19{\cdot}4 - 0{\cdot}47x)$.

Department of Pathology,
University of Sheffield Medical School,
Beech Hill Road,
Sheffield S10 2RX, U.K.

T. J. STEPHENSON
D.W.K. COTTON

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Sweat pores on fingertips

MADAM, I was interested to read the paper by Bachmann Andersen and Thestrup-Pedersen (*British Journal of Dermatology* 1987; 117: 225) and I would like to comment on it.

It is well-recognized that epidermal ridges are, on the whole, wider in males than in females and differences are only partly accounted for by the larger hand size in males.[1] It has been reported that averaging values for various parts of the palm, including the fingertips, in adults, gave a mean of 20·7 ridges/cm in males and 23·4 ridges/cm in females.[2] Thus, it would be expected that pore counts per unit area of fingertip would be higher in females than in males. Moreover, the distances between successive sweat pores on a ridge are usually about equal to the width of the ridge[3] and this would explain the significantly increased number of pores per unit area in females.

Thus, the tendency to wider ridges in males is more important than the larger hand.

The finding of flattened epidermal ridges in atopic eczema is also not new; I described it some years ago.[4,5]

Department of Dermatology,
Royal Liverpool Children's Hospital,
Myrtle Street,
Liverpool,
L7 7DG, U.K.

J. VERBOV

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MADAM, Bachmann Andersen and Thestrup-Pedersen (*British Journal of Dermatology* 1987 117: 225) report that sweat pore density on the fingertips is greater in women than men, and that there is a higher density of sweat pores on the third and fourth fingers. They conclude that the reason for this is obscure.

Szabo,[1] almost 25 years ago, argued convincingly that a major factor in the density of sweat glands was the relative change in surface area of differing parts of the body with growth and we have used this hypothesis to explain differences in sweating between children and adults.[2] It comes as no surprise, therefore, that the density of sweat pores on the fingers is inversely proportional to finger size. The authors state that the areas of fingertips 1,2,3 and 4 are the same, but provide no evidence to justify this claim, nor is the surface area of the tip, however defined, the relevant factor rather than say the area of the whole finger.

The authors also report consistent differences in density between atopic men and control subjects although they claim the differences are not statistically significant. Unfortunately, the results are presented in such a fashion that it is not possible to assess the power of the statistical testing (i.e. the possibility of a type 2 error). Can the authors oblige?

University Department of Dermatology,
Royal Victoria Infirmary,
Newcastle upon Tyne, U.K.

J.L. REES

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Reply

MADAM, Thank you for comments from Dr Verbov and Dr Rees regarding our investigation of sweat pore density on the fingertips of atopic patients. We apologize to Dr Verbov that neither we nor our computers were aware of his excellent studies published in 1970 and 1972. These investigations, however, deal with counting of whorls, loops and arches on the palms and fingers of patients with atopy and controls. Counting of sweat pores was not performed. Both Dr Verbov's and Dr Rees' explanations of our finding of a difference in sweat pore number between males and females are valuable comments for the reader. We can inform Dr Rees that we did not make exact measurements of the total areas of fingertips due to technical difficulties. As stated in our article, we counted the number of sweat pores in a standard area in the centre of each finger pulp. The individual results in atopic men and control subjects were not presented due to limitations of space. We used non-parametric statistical methods as stated and failing to show statistical significance given the number of persons studied, we do not feel confident that there is a real difference between atopic and non-atopic men although such a difference may indeed exist.

Thank you for making us and other readers aware of important work and especially of an explanation for the difference between the sweat pore density in men and women.

Department of Dermatology,
Marselisborg Hospital,
8000 Aarhus C,
Denmark

L. BACHMANN ANDERSEN
K. THESTRUP-PEDERSEN

Expression of involucrin in skin appendage tumours

MADAM, We read with interest the recent article by Hashimoto *et al.* (*British Journal of Dermatology* 1987; **117:** 325) concerning the expression of involucrin in skin appendage tumours. We would like to point out that we have already published an article on this,[1] which was not cited by these authors. The results obtained by Hashimoto *et al.* although generally similar to ours, differed in some aspects. In agreement with previous studies[2] we did not observe any involucrin expression in the secretory portion of either eccrine or apocrine sweat glands, structures that do not undergo terminal keratinization. This finding is corroborated by the lack of reactivity for involucrin found both by us and by Hashimoto *et al.* for tumours (such as hidradenomas) that are considered to exhibit to a variable extent a secretory derivation or differentiation.[3] Conversely, we found a positive reaction for involucrin in syringomas, which are dilatations of the dermal sweat-duct, i.e. of a structure normally expressing involucrin. These results show that involucrin expression in adnexal proliferations of the skin parallels that observed in the normal counterparts of the cutaneous adnexa, and therefore we agree with Hashimoto *et al.* that immunostaining for involucrin provides useful information in the immunohistochemical investigation of cutaneous tumours.

Clinique Dermatologique
Hopital Edouard Herriot,
69434 Lyon Cedex 3, France

J. KANITAKIS

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Reply

Thank you for letting us see the letter by Dr Kanitakis. We would like to reply to it as follows. First of all, we must apologize to Dr Kanitakis for failing to cite their article in our paper. This is simply because Virchows Archive did not appear in the literature survey when our manuscript was written.

In our study, positive staining for involucrin was consistently found on the luminal surface and the canaliculi of the secretory portion of eccrine sweat glands, which differs from the results obtained by Kanitakis *et al.* and Murphy *et al.*[1] We consider it possible that a small amount of involucrin is produced in the inner cells of the secretory portion and accumulates at the luminal surface.

As mentioned in our article, involucrin expression was not consistently observed on the luminal surface of the secretory portion of ducts or apocrine sweat glands. This variability in staining was also observed in tumours of apocrine sweat gland origin, since involucrin was expressed in apocrine cystadenoma, but not in Fox-Fordyce disease. Most interestingly, in specimens from two cases of extramammary Paget's disease, one was stained and one was not. A similar result was obtained by Kanitakis *et al.*

As far as syringoma is concerned, we also expected positive involucrin expression, as Dr Kanitakis suggested. The failure of staining might be due to the different site of the tumour, because in our case the syringoma appeared in the genital region.

Generally, in tumours of the hair follicle, consistent staining for involucrin was obtained, similar to that in normal hair follicles. However, in sweat gland tumours, involucrin expression was somewhat inconsistent, which means that more cases need to be studied before a final conclusion can be drawn. A similar study has recently been reported by Mikami *et al.*[2]

Section of Dermatology,
The Kitasato Institute Hospital
Tokyo 108, Japan

T. HASHIMOTO

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PMN migration in psoriasis

MADAM, We were interested to read the article by Pease *et al.*, (*British Journal of Dermatology* 1987, **117**, 161) who investigated polymorphonuclear leukocyte migration in patients with psoriasis vulgaris. The authors found no differences between psoriasis patients and age and sex-matched control individuals in PMN responses to FMLP, psoriasis serum or zymosan activated serum. The authors discussed their negative finding in the light of recent conflicting reports on functional abnormalities of PMN in psoriasis.

We feel that the report by Pease *et al.* not only provides additional data on PMN function in psoriasis, but also raises the important question of how to define 'disease severity'. In the report by Pease *et al.*, patients with 'mild' psoriasis (5–20% body surface involved) and 'severe' psoriasis (more than 40% body surface involved) were grouped for comparison, disease severity thus being defined according to the extent of body surface involvement. Although this way of looking at psoriasis is used in the literature, it may cause confusion in interpreting experimental data as well as misunderstanding of the disease. In previous reports[1–6] we have pointed out 'that PMN functional abnormalities including chemotaxis and enzyme degranulation in response to various chemotaxins (e.g. the complement component C_{5a}, leukotriene B_4, FMLP as well as casein or autologous serum) 'can be seen in patients in which skin lesions are still developing or show rapid growth'. The state of active disease is paralleled by increased functional responses of psoriatic PMN to known chemotaxins.[5]

By measuring the duration of the latest relapse it was found that in patients with eruptive psoriasis dating back not more than 2 months, PMN chemotaxis was significantly increased compared with that in patients with disease onset dating back 3 to 5 months or more than 6 months ($P < 0{\cdot}001$).[5] However, when patients in remission, without active disease, were studied, PMN chemotaxis in patients who had had 20–50% or even above 50% body surface involvement did not differ from that in normal controls.

These as well as other reports indicate that, in psoriasis, changes in PMN functional activity are more probably related to the activity of the disease than to the extent of body surface involvement. This appears to be of interest since it may throw some light on trigger mechanisms involved in the psoriatic tissue reaction. We feel it necessary not to overlook this aspect when leukocyte studies in psoriasis are performed. In fact it appears not to be helpful to publish such data without considering the disease activity.

Department of Dermatology and Venerology
Christian-Albrechts University,
Schittenhelmstr. 7, 2300 Kiel, Germany

E. CHRISTOPHERS
A. LANGER*

* Department of Dermatology,
Warsaw Academy of Sciences,
Warsaw, Poland

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Reply

MADAM, The question of how best to define disease activity in psoriasis or indeed in patients with psoriatic arthritis is important. A recent worsening of their disease is clearly relevant when it comes to defining disease activity. Although we described the patients in our severe psoriatic group by the percentage of the skin involved, nine out of the 10 in fact had skin disease which was getting worse. Likewise four of the eight patients with psoriatic arthritis had active skin disease while four of the 16 patients with mild psoriasis (i.e. under 20% of body surface involved) had reported a 'recent worsening' of their condition, although in no case was this severe. However, I did not record in each case the duration of this relapse. Thus, I cannot comment on the polymorph responsiveness in patients with eruptive psoriasis dating back not more than 2 months. In Figure 1, which is taken from our paper, I have joined the results for those patients with psoriasis which was getting worse (or 'active') with the results for their paired age and sex-matched controls. The results do not demonstrate any discernible pattern. In view of this data I came to the

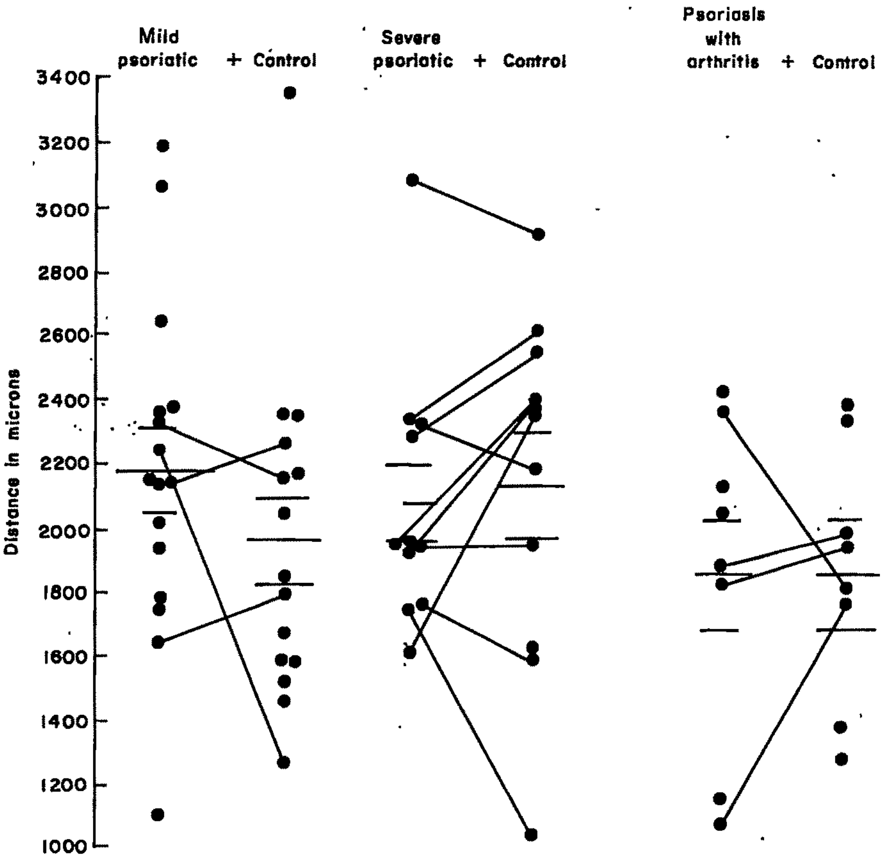

FIGURE 1. Directed PMN motility from patients with mild or severe psoriasis or psoriatic arthritis compared with their respective controls. FMLP was used as chemoattractant. Bars represent means ± SEM. Lines connect results for patients whose psoriasis was getting worse with their respective age and sex-matched controls.

conclusion that there was not a primary abnormality of polymorph function in patients with psoriasis vulgaris.

Department of Immunology and Rheumatic Diseases,
Charing Cross Hospital (Fulham),
Fulham Palace Road, London W6 8RF, U.K.

C.T. Pease

Unilateral hyperhidrosis

Madam, Dr Van De Kerkhof and his colleagues discussed unilateral hyperhidrosis in their recent article (*British Journal of Dermatology* 1987; 117: 779).

I would draw attention to this symptom as a rare presenting manifestation of Buerger's disease. Some years ago a man in his late twenties, who was a heavy smoker, presented with gross unilateral hyperhidrosis affecting one leg and foot. There were no other symptoms, but within 6 months intermittent claudication began followed by thrombophlebitis migrans and the diagnosis became obvious.

Dermatology Department
The London Hospital
Whitechapel, London E1 1BB, U.K.

H. BAKER

Career prospects in dermatology

MADAM, I have just reread through my copies of the BJD prior to their going to the binders. I was particularly interested to reread a letter by Dr Meyrick Thomas and his colleagues (*British Journal of Dermatology* 1987; **116:** 284). The subject of this letter was the poor career prospects in dermatology in the United Kingdom. Of the 20 signatories of this letter it would appear eight have now obtained consultant appointments. Of the others, some, such as Dr Pegum and the registrar signatories, would perhaps have not been expected to have obtained appointments during the last year. I wonder if this could be taken as evidence that career prospects in dermatology are not in fact all that bleak.

Department of Dermatology
Central Out-Patients Department
Hartshill Road, Stoke on Trent ST4 7PA, U.K.

A.G.SMITH

British Journal of Dermatology (1988) 118, 590–596

Book Reviews

Models in Dermatology. Edited by H.I.MAIBACH AND N.J.LOWE, Vol 3 (1987) Basel. Karger. Pp 203. Price £113.70.

Forming the third in a series, this multi-author book follows a similar format to that of its two earlier companion volumes. The chapter headings indicate the overall breadth of coverage; sections covered include cell biology, dermatopharmacology, dermatotoxicity, metabolism, percutaneous penetration, physiology, immunology, disease models, microbiology and wound healing.

Most of the contributions are lucid and well referenced having predominantly a 'basic science' approach, although there are a few clinically orientated contributions; clinical assessment of acne vulgaris, evaluation of topical antipruritics and measurement of cutaneous irritant reactions.

For readers with an area of interest covered by the book, it will prove an excellent source for further references and the inclusion of a cumulative index for all three volumes facilitates such information retrieval.

This book, like the two previous volumes, will no doubt prove to be popular with academic departments, but in spite of the excellent quality paper used, enabling high quality reproduction of monochrome (no colour) photographs, the selling price of approximately £110 for this single volume really does seem excessive. Perhaps a single coherent volume logically laid out and avoiding duplication would be much more successful commercially and permit a more reasonable price tag.

D.M.MACDONALD

Life-threatening Dermatoses. P.A.KRUSINSKI AND F.P.FLOWERS (1987). Chicago: Year Book Medical Publishers, Pp 300. Price £38.50.

This book, from the Year Book Medical Publishers, is a sequel to *Dermatology in Ambulatory and Emergency Medicine* and was written, the authors say, because of the great interest aroused by their chapter in it on life-threatening dermatoses. This interest is hardly surprising from those inside and outside dermatology who are reminded that patients with skin disease can die, but can also be saved if dealt with skilfully, some of the skills being special to our field.

The first nine chapters deal with clinical syndromes and the 10th, on algorithms and principles of clinical decision-making, is perhaps least likely to be read in detail. Algorithms are used throughout, at the ends of the clinical chapters: they are worth persevering with here because, as clinical flow charts, they provide a useful summary of the diagnostic and management aspects of each disease group. These are diffuse erythemas, collagen vascular diseases, vesicobullous diseases, life-threatening pustular dermatoses, purpuric and haemorrhagic disorders, opportunistic infections and acquired immunodeficiency syndrome, melanoma and its precursors, paediatric life-threatening dermatoses and erythematous nodules. Thus, with the exception of the chapter on melanoma, the book stresses the role of dermatologists as specialist physicians. Some of the conditions described are obviously more immediately life-threatening than others and some, like brown recluse spider bites, though of interest, are not likely to be of much practical importance in the U.K. The 11 contributors are American, so it is hardly surprising that the references are biased towards work from that side of the Atlantic. There is little overlap or contradiction and the advice given is for the most part practical, clear and, when it needs to be, dogmatic.

This is a book for dermatologists and many other hospital specialists to buy. Once it is bought it will be much used.

JANET M.MARKS

Dermatology Drug Directory. V.N.SEHGAL AND G.SRIVASTAVA (1987) New Delhi: Jaypee Brothers, Medical Publishers. Pp 128. Price Rs. 45.00.

This unusual handbook is designed to help Indian practitioners care for their patients suffering from skin and venereal diseases, as well as leprosy. Some of the enthusiasm and individuality of the Indian Association of Dermatology, Venereology and Leprosy rubs off into it.

Unfortunately, the authors start with the assumption that skin diseases are easily diagnosed by family doctors, and list treatments of them without a balanced or critical approach, or a sense of proportion. A remarkable number of possible side-effects of systemic drugs occupies much of the text without any indication of their frequency or severity. A number of treatments mentioned lack reason—vitamin K5 for alopecia, griseofulvin for lichen planus, and some are completely bewildering, such as a course of metronidazole, erythromycin, mebendazole and diethyl carbamazine in chronic urticaria. No wonder the cover design is stated to depict 'a dazed doctor unable to manage the disease'. Perhaps he has just read page 72 listing drugs causing 'puerpuric eruptions'. Alas, spelling errors are on every page. It is a shame, but the information in the Directory must be treated with caution.

R.R.M.HARMAN

Pharmacology and the Skin, Vol. 1. Skin Pharmacokinetics. Edited by B. SHROOT AND H. SCHAEFER (1987) Karger, Basel. ISBN 3-8055-4555-X. Pp 264. Price £90.

This pleasantly produced book is in fact the proceedings of the 7th CIRD Symposium, held in September 1986. The text is divided into five main sections, beginning with the structure and function of permeability barriers which contains an excellent review by Elias *et al.* on the stratum corneum two compartment model. The second section is on general principles of skin permeability and includes a chapter on skin and nail penetration and one on 'reverse permeability', looking at griseofulvin in the skin after oral administration. The third section deals with the continuing difficulties with models of percutaneous absorption and the remaining two sections are devoted to variables in skin permeability and practical applications of skin pharmacokinetics. The last 40 pages give details of nine poster presentations.

It is difficult to be confident that any material found in this text has not been or will not be published elsewhere, and in view of the high price, prospective purchasers may wish to obtain a copy on approval before they make the decision to part with £90.

RONA M.MACKIE

Dermatology in General Medicine, 3rd Edition. T.B.FITZPATRICK, A.Z.EISEN, K.WOLFF, I.M.FREEDBERG AND K.F.AUSTEN (1987) New York: McGraw-Hill. Pp 2641. Price £160.

The 3rd (green) edition of *Dermatology in General Medicine* has two volumes, seven parts (introduction, biology and pathophysiology of skin, disorders arising in the skin and mucous membranes, dermatology and internal medicine, disorders due to microbial agents, therapeutics and paediatric dermatology), 34 sections and 223 chapters. There are just under 250 contributors, some 100 of whom are not based in dermatology departments.

Nearly all of the international band of authors have written or carried out research on their respective subjects. In places their enthusiasm knows no bounds and some might consider the space allocated to the various chapters uneven (e.g. porphyrias, 48 pages and 450 refs; disorders of pigmentation, 82 pages and 575 refs; benign neoplasias and dysplasias of melanocytes, 69 pages and 558 refs; psoriasis, 30 pages and 175 refs; atopic dermatitis, 24 pages and 227 refs; lupus erythematosus, 18 pages and 44 refs). I take the other view and would be sorry to see some of these masterly chapters shortened, the more so as many are probably the best reviews of the subjects available at present. The section on biology and pathophysiology of skin (368 pages) is especially good and is, in itself, a remarkably comprehensive and up to date account of the state of the art which, again, has few rivals. The black and white photographs and line diagrams in the book are generally good. The colour atlas, repeated at the beginning of each volume, includes many over-vivid pictures; they add a little, but not much, to the text.

Much of the book could and should be read regularly by all dermatologists as its educational value is enormous. Those practising in other branches of medicine will find it to be most useful for reference. Although it is not as orderly as the Rook book and does not cover as many dermatological syndromes and minutiae, it contains chapters which deal with some subjects in a broader and more critical way than its rival. It also includes much information on purely dermatological conditions (from Kyrle's disease to lichen nitidus), a point belied by the title of the book.

In the last few months I have been lucky enough to review both the Rook and Fitzpatrick books for this journal. After spending many hours reading sections from each I will never be in a better position to advise readers of the *British Journal of Dermatology* which to buy. They are very different but, without doubt, complementary. Dermatologists and libraries should not choose one or the other; they will need both.

J.A.A.HUNTER

Androgen Metabolism in Hirsute and Normal Females. Edited by R.HORTON AND R.A.LOBO. 1986. Clinics in Endocrinology and Metabolism Vol 15, No 2. London: Saunders. Pp 213 to 409. Price £15.00.

I found this book extremely informative and helpful. It provides a wide ranging view of androgen metabolism in women, both in normal women and in the pathological states of hirsutism, polycystic ovary disease and acne.

This is a multi-author book and because of this many of the views given are those of the individual authors; thus both sides of certain controversies are presented, e.g. the role of free and bound androgens in determining tissue availability. This is a strength of the book as it avoids the oversimplification that often occurs when a subject is addressed by a single author.

The biochemical sections of this book will be of great value to anyone who is already interested in the field of androgen metabolism in women or who requires to become acquainted with the literature. The chapters are informative and give detailed references, thus providing a ready 'handle' on the literature. The clinical sections also provide a useful summary of some of the current state of the art in the therapy of androgen-related disorders.

I have no hesitation in recommending this book to anyone with an interest in androgen metabolism in women.

FENELLA WOJNAROWSKA

Pathobiology of Malignant Melanoma Edited by D.E.Elder. Vol. 8 in Pigment Cell, Ed. R.MACKIE (1987) Basel: Karger, Pp 225. Price £79.10.

This volume is an excellent and up-to-date review of current thinking on many aspects of the pathobiology of malignant melanoma. It cannot be regarded as a complete melanoma reference text, but concentrates rather on difficult aspects of tumour developmental biology that are often neglected, but are so essential to early and correct diagnosis.

Christian Schmoeckel, in his chapter on Diagnosis of Early Malignant Melanoma, points out the lack of consensus even among acknowledged experts on histological criteria for diagnosing early malignant melanoma. In most centres throughout the world more cases of early malignant melanoma and atypical naevi are being seen and it is only by painstaking research and careful clinicopathological correlation involving large numbers of lesions and patients that we will ever become more adept at the diagnosis of such lesions. The contributors to this book are all very well equipped to undertake such studies and their writing reflects not only great experience, but also (in most cases!) clear thinking. The Editor, David Elder, together with Wallace Clark, has contributed two key chapters on the developmental biology of malignant melanoma, and tumour progression and prognosis. Their accounts are scholarly and very readable. Bernard Ackerman provides a stimulating chapter discussing what he admits is his 'near obsession with the subject of malignant melanoma in situ'. The essay is cogently written, although some workers may find Dr Ackerman's histopathological criteria for melanoma in situ equally applicable to some dysplastic naevi. Peter Heenan and colleagues contribute a very useful review of melanoma variants and Richard Sagebiel lucidly discusses the important issues of tissue processing and microstaging of melanoma—areas that are so often taken for granted. Other excellent chapters cover laboratory studies in patients with the dysplastic naevus syndrome, the cell biology of melanocytic tumours and aspects of metastatic melanoma.

On a personal note, I would very much like to have seen included in this volume a chapter by Richard Reed who has contributed so much to our understanding of the pathobiology of melanocytic tumours. The book as it stands, however, is a truly excellent one and admirably complements Volume 6 in the same series (also on melanoma and edited by Rona MacKie). I can genuinely say that of all the books on malignant melanoma currently available I have found this volume the most rewarding and stimulating. Go out and buy a copy today!

N.SMITH

Pigmented Lesions of the Skin J.C.MAIZE AND A.B.ACKERMAN (1987) Philadelphia: Lea & Febiger, Pp 328. Price US $98.50.

'Clinicopathologic correlations' is the sub-title of this beautifully produced volume and the authors' objective is 'to provide clinicians and pathologists with a concise guide to the definitive diagnosis, differential diagnosis and biologic course of pigmented lesions in the skin'. Despite the enormity of this task that the authors have set themselves, they must be congratulated in largely achieving their objectives. The book is copiously illustrated with clinical photographs, histological photomicrographs and, where appropriate, excellent line drawings. The chapters are organized in a logical sequence starting with a definition of terms and general principles and progressing through sections on biopsy and benign pigmented lesions to malignant melanoma, its histological simulants and non-melanocytic pigmented lesions. Each entity is defined, its clinical and histological features are described and there then follow sections on clinicopathological correlations, biological behaviour, and special studies. This structure works extremely well and most of the numerous photomicrographs are truly superb. Selected references are given at the end of each chapter.

All the sections are well written, in particular the section on histological simulators of malignant melanoma covers an important area for the pathologist. In this section I feel it is a pity that the pigmented spindle cell tumour of Reed is relegated to the status of a variant of Spitz naevus. More importantly it has to be stated that the authors' concept of the histopathology of dysplastic naevi is quite different from that of Drs Elder, Clark and others from the Philadelphia group who have carefully reported their criteria over many years in many papers. More experienced readers will be aware of this, but one suspects that the book will be bought by many doctors in training with the result that we may have a large increase in the numbers of dysplastic naevi diagnosed in our patients!

This caveat aside, 'Pigmented Lesions of the Skin' can be heartily recommended. It should genuinely appeal to both dermatologists and pathologists, especially those in training. By using well-defined and reproducible diagnostic criteria, good quality illustrations and a well thought out structured approach, Drs Maize and Ackerman have made a major contribution to a difficult and often confusing subject.

N.SMITH

Immunopathology of the Skin E.H.Beutner, T.P.Chorzelski and V.Kumar (1987) 3rd edition. Chichester, John Wiley. Pp 769, Price £78.

The third edition of *Immunopathology of the Skin* focuses on skin diseases, particularly bullous and connective tissue diseases, in which immunofluorescence and other laboratory methods are of diagnostic value. The book has been written primarily for immunopathologists and dermatopathologists, but also aims to provide a reference for general pathologists and dermatologists. This small, compact book is undoubtedly packed with information, of value to both groups. The text is concise, with organization into four sections: basic concepts and methods, vesiculo-bullous diseases, connective tissue diseases, and other dermatoses with significant immunohistological findings. The text has been considerably expanded and updated since the second edition, including details of new techniques and recent advances in elucidation of the pathogenesis of skin diseases.

The first section deals with methodology in considerable detail, including immuno-electron microscopic techniques, and would be particularly valuable to those whose laboratory is venturing into the field of cutaneous immunopathology for the first time. This section also includes a succinct chapter on autoimmunity, which provides a useful framework for interpretation of immunological responses described in later chapters.

Sections 2 and 3 deal with the skin disorders in which the role of immunopathology is well established, but provide far more information than earlier editions about the reliability of techniques and interpretation of results. Immunopathology of the connective tissue diseases has been usefully expanded, with the addition of new chapters dealing with neonatal lupus and Sjogren's syndrome.

Section 4 is a particularly welcome expansion, which answers many diagnostic queries for those employing immunopathological techniques in a routine histology laboratory. Thus the significance of keratinocyte cytoplasmic and stratum corneum antibodies is discussed. Graft versus host disease and wart virus infection are included for the first time.

Although not intensively illustrated this book contains ample, high quality black and white photographs, which clearly depict the positive immunofluorescence results. The book is also extremely well-referenced, with numerous and reasonably up to date references at the end of each chapter.

If there is one criticism, it is that of irritation at the excessive use of abbreviations. This is, undoubtedly, a transatlantic custom and perhaps acceptable or desirable to our American colleagues. However, the average British reader, who is not deeply involved in research in this field, may find the text loses fluidity in places because of the necessity to refer back to the beginning of the book, where a total of 271 abbreviations are listed.

This reasonably concise volume admirably fulfils the authors' ambitions and can be recommended to both dermatologists and pathologists.

Janice Anderson

The Dermatology of Bacterial Infections. G.H.Findlay (1987) Oxford: Black Scientific Publications, Pp 370, Price £49.50.

George Findlay writes as a dermatologist with considerable practical experience of treating bacterial infections and as one who has read widely on the subject. The format, he tells us in his preface, is intended to simulate the seminars which he has given in the University of Pretoria over many years. The main text is in continuous prose and is readable if somewhat discursive; in addition, and punctuating it, are boxes in which the essential features of different infections are set down in tabular form. In other boxes there are lists of aphorisms from various sources; some are acknowledged, some are presumably Findlayisms.

The book is organized on historical lines, something of a curiosity among texts concerned with microbiology these days. The first section is therefore devoted to the well known Classical Infections starting with A for anthrax, B for boils, etc. Then comes Hospital Infections, with Semmelweis and

Florence Nightingale featuring prominently. Towards the end we get Newer Infections which include the staphylococcal scalded skin syndrome and Lyme disease, but before that The Intermediate Era features a whole range of pathology: pseudomonas infections, Haverhill fever, and trichomycosis axillaris, to list but a few.

The book is extremely comprehensive and almost always the approach to each infection is the historical one. Taken individually these accounts of diseases are informative and practical, but I doubt whether such an approach is really sustainable through a whole textbook.

Professor Findlay gives us only a small number of references for further reading; a perfectly legitimate device in a short textbook and indeed in seminar teaching. But *The Dermatology of Bacterial Infections* runs to 370 pages and the author has such fascinating tales to tell that the reader will, I suspect, feel frustration in not having direct access to some of his intriguing sources.

While acknowledging that it is a major sin for a reviewer to complain that the book before him is not the text the author 'should have written' it does seem a pity that Professor Findlay was not persuaded to eschew his boxes and his punchy accounts of bacterial infections and encouraged to write us a purely historical text. However, the volume he has written is one which dermatologists with a microbiological bent will want to explore and will do so with profit and enjoyment.

S.O.B.ROBERTS

Colour Aids: Dermatology. J.D.WILKINSON, S.SHAW AND D.A.FENTON (1987), Edinburgh: Churchill Livingstone, Pp 154, Price £5.95.

This small and relatively inexpensive volume is essentially a colour atlas of common dermatological conditions with accompanying short notes on aetiology, clinical features and treatment. The strength of the book lies in its 235 excellent photographs and there is no doubt that it will be of great help to students or examination candidates who often have only limited exposure to dermatology in the undergraduate course. The format, although convenient, means that the notes for each condition are necessarily extremely brief and for this reason the book is no substitute for a dermatological textbook. However, many textbooks have few photographs and this book, if used in association with a conventional text, will help to make up for this deficiency.

As always one has a few quibbles. Figure 99 which purports to show 'giant urticaria' would be more accurately labelled 'generalized urticaria' like the very similar Figure 65, and I suspect that the term 'Tycoon Scalp' (Fig. 87), is not one that would be recognized by the majority of dermatologists. Although the book has a table of contents it is quite difficult to find a particular condition quickly and I think it would benefit from an index.

In general, this is a very welcome addition to student textbooks and the authors and publishers are to be congratulated on producing it at such a reasonable price.

R.D.G.PEACHEY

Oxford Handbook of Clinical Specialities. J.A.B.COLLIER AND J.M.LONGMORE. (1987) Oxford: Oxford University Press, Pp 787, Price £9.95.

This is, by any standards and whatever your opinion, an extraordinary little book. It is a sequel to the very popular *Oxford Handbook of Clinical Medicine* (or OHCM as the authors call it). I asked our medical students what their opinion was of the original OHCM and they told me that everyone had bought one, and that they thought it was marvellous. Many had not really had a chance to assess this new volume properly, but most considered that the concept was quite a good one.

The new book contains nine speciality chapters: gynaecology, obstetrics, paediatrics, psychiatry, general practice, ophthalmology, ear, nose and throat diseases, dermatology and orthopaedics/rheumatology/trauma. There is also a section on eponymous syndromes and diseases. The authors outline their philosophy and their reasons for writing it in an introductory section and also in an amusing little tailpiece (which is certainly worth a read!). They are concerned that 'the great tomes which mark the road to knowledge can numb the mind after a while'. They hope that this book will act as an introduction to the specialities and that it will be discarded in due course.

I cannot really comment on the other speciality sections and I have, therefore, tried to look at this book from the specific viewpoint of the teacher of undergraduate dermatology. In particular, I tried to assess whether or not I would want our students to use it.

Dermatology is 'covered' in 12 pages, each only 17 by 9 cm. What has been included for each of the specialities has been reviewed by a relevant authority and dermatology is no exception. It is, therefore, hard to argue with the facts that are presented, but I was sad to see the old dermatological howler 'pruritis' on the differential diagnoses page of the skin diseases section. As can be imagined, 12 small pages only give room for a text which is extremely spare and limited, and there is, in my opinion, insufficient flesh to put the information provided into a proper clinical context. I would also be concerned that this book, once purchased, would remain undiscarded in favour of large texts, except by those who go into the individual specialties themselves. My recommendation to our medical students will, therefore, be to read this book by all means, but also to buy one of the excellent smaller British textbooks of dermatology which will, I believe, provide a much more satisfactory introduction to our specialty.

R.GRAHAM-BROWN

Thesis received

Skin cancer and UV exposure: Investigations on the estimation of risks. H.Slaper (1987) Dept of Dermatology, State University Hospital, Utrecht, The Netherlands. Pp 158.

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News amd Notices

European Society for Dermatological Research, Annual Meeting

The annual meeting of the European Society for Dermatological Research will be held in the Arabella Conference Center, Munich, West Germany, from 19–22 June 1988. Further details available from: Professor Dr Johannes Ring, Department of Dermatology, University of Munich, Frauenlobstrasse 9–11 D-8000 Munchen 2, West Germany. Tel 089/5397-660/624.

Advance Meeting Announcement
A Century of Porphyria

A specialized meeting entitled 'A Century of Porphyria' will be held in the Department of Medicine, Western Infirmary, Glasgow, U.K. from 4–7 April 1989. The meeting will be held in conjunction with the Tetrapyrrole Discussion Group (U.K.) and will be dedicated to the Head of Department, Professor Sir Abraham Goldberg, on the occasion of his retiral. For further details contact the Meeting Coordinator, Dr M.R.Moore, Department of Medicine, Gardner Institute, Western Infirmary, Glasgow G11 6NT, U.K.

Practical Skin Pathology Course

A course on Practical Skin Pathology accredited by the American Medical Association and the American Academy of Dermatology will be given in Amsterdam, The Netherlands, 11–14 July, 1988. This is a rigorous, comprehensive, practical review and update of dermatopathology for pathologists and dermatologists. Microscopes and extensive syllabus material will be provided. There will be 24 formal lectures and daily supervised microscope sessions of 400 unknown workshop slides. The course directors are Martin H. Brownstein (New York), Chris J.L.M.Meijer (Amsterdam) and Theo M.Starink (Amsterdam).

For further information, please contact: Martin H.Brownstein, M.D., 2 North Plandome Road, Port Washington, NY 11050, U.S.A. Tel: (516) 944-3883; or Theo M.Starink, M.D., B.v. Zuylenlaan 11, 1183 EE Amstelveen, The Netherlands, Tel: (020) 548-3496.

Third International Symposium on Paediatric Dermatology
Alteration in Arrangements

The third International Symposium on Paediatric Dermatology, to be held in Mazara del Vallo, Trapani, Italy, will take place on 14–17 September 1988 and not on 21–24 September as announced in the October issue.

European Academy of Dermatology and Venereology

The European Academy of Dermatology and Venereology (EADV) was founded on Saturday, 3 October, 1987, in Luxembourg in the Bâtiment Jean Monnet of the European Economic Community by representatives from Belgium, Denmark, France, Great Britain, Greece, Ireland, Italy, Luxembourg, the Netherlands, Portugal and Spain and with observers from Germany.

The main aims of the EADV are to provide continuing medical education and up-to-date information of interest to European dermatologists and venereologists and promote the exchange of practical and scientific information. Initially the means for achieving these aims will be a biennial European congress and an information bulletin.

The officers of the newly formed European Academy of Dermatology and Venereology are: President: Emiliano Panconesi, M.D.—Florence, Italy; Vice President: Philippe Lauret, M.D.—Rouen, France; Secretary General: Hans Rothenborg, M.D.—Hellerup, Denmark; Treasurer: Michel Delune, M.D.—Brussels, Belgium; President Elect: Nicholas Parisis, M.D.—Athens, Greece. The first EADV congress will be held in Florence, Italy from 25 to 28 September 1989.

Second National Congress of the Uruguayan Society of Dermatology

The second National Congress of the Uruguayan Society of Dermatology will be held at the Convention Centre, City Hall, Montevideo, Uruguay, from 30 October to 1 November 1988. The official topics of the Congress will be cutaneous granulomas and genodermatosis. For further information contact: Dr Raúl Vignale, Meetings S.R.L., Av. 18 de Julio 1268 Of 601, Montevideo, Uruguay. Telephone: 91 52 31.

International Symposium on Cutaneous Lymphoma

This symposium will be held in Copenhagen on 28 to 30 October 1988. Topics will include tumour immunology, cell adhesion, retroviruses, diagnostic techniques and therapy. Free communications are welcome. For further information contact: Gunhilde Lange Wantzin MD, Bispebjerg Hospital, Dept of Dermatology, Bispebjerg Bakke 23, DK 2400 Copenhagen N.V. Denmark.

Advance notice: Institute of Dermatology
Clinical Dermatology in the Year 2000

This International Symposium (President: Arthur Rook) will be held in the Barbican Centre, London 22–25 May 1990. The purpose of the Symposium is to define expected major advances in the practice of dermatology during the last decade of the century. All major aspects of diagnosis and treatment will be covered by a faculty of international experts. Further details from: Professor Malcolm Greaves, Institute of Dermatology, St Thomas' Hospital, Lambeth Palace Road, London SE1 7EH, U.K.

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Eosinophil chemotactic factor in tumour tissue from a patient with Kimura's disease

M.ISODA

Department of Dermatology, Hiroshima Red Cross Hospital, Hiroshima, Japan

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SUMMARY

An extract of tumour tissue from a patient with Kimura's disease was tested for eosinophil chemotactic activity using the Boyden chamber method. Eosinophil chemotactic activity was detected in the tissue extract, and after gel filtration maximum activity was found in the fraction with a molecular weight of approximately 1000 daltons. This factor may play a role in the tissue eosinophilia which occurs in Kimura's disease.

Kimura's disease is characterized clinically by marked eosinophilia in the peripheral blood and asymptomatic subcutaneous tumours, and histologically by follicular aggregations of lymphocytes with well-developed germinal centres, and marked eosinophilic infiltration. The disease is rare, being seen only in Orientals. Subcutaneous angiolymphoid hyperplasia with eosinophilia (ALHE), reported in the West,[1] has been used synonymously with Kimura's disease.[2–5] However, its relationship to Kimura's disease is still controversial.[6,7]

Tissue eosinophilia in this disease is thought to be the result of an IgE mediated immune response, because of elevated serum IgE levels, infiltration of eosinophils and mast cells into the tumours, and detection of IgE in the lymphoid follicles on immunofluorescence.[8] However, there are no reports to our knowledge about eosinophil chemotactic activity in the tissue.

The present study was undertaken to examine the eosinophil chemotactic activity of tissue from a typical case of Kimura's disease.

CASE REPORT

A 19-year-old man was hospitalized in March 1984 because of a solitary, asymptomatic, subcutaneous tumour in the left preauricular area. The tumour had first appeared when the patient was 6 years old and despite resection, cobalt 60 and soft X-ray irradiation, and

Correspondence: Midori Isoda, Department of Dermatology, Hiroshima Red Cross Hospital, Senda-machi 1, Naka-ku, Hiroshima 730, Japan.

prednisolone therapy, had recurred several times. He had no history of atopic disorders. Physical examination disclosed a firm skin-coloured, subcutaneous tumour, 45 × 70 mm, in the left preauricular area. The regional lymph nodes were not enlarged. Remaining physical examination was normal. A full blood count was normal except for a white cell count of $11{\cdot}2 \times 10^9$/l, with 21% eosinophils. No atypical lymphocytes were seen. Liver function tests, serum electrolytes and serum protein electrophoresis were all within normal limits. Urinalysis showed occult bleeding, due to horseshoe kidney, which had been confirmed by intravenous pyelography 6 years earlier. The serum IgE level was 2000 IU/ml (normal, <500 IU/ml) measured by radioimmunoassay. The tuberculin skin test and immediate cutaneous reaction to *Candida* antigen were positive (14 × 11 mm erythema). Serum anti-*Candida albicans* IgE antibody measured by RAST was negative.

A biopsy specimen from the left preauricular tumour showed many lymphocytic aggregations with germinal centres in the dermis and subcutaneous fat, with moderate eosinophilic and histiocytic infiltrates.

The tumour was resected together with part of the left parotid gland, but some of the tumour remained in the deeper tissue. One month after the operation, the patient was started on prednisone, 30 mg/day. Three months after admission, the tumour had regressed slightly and the patient was discharged.

METHODS

Extraction of putative chemotactic factor from the tumour tissue

A surgical specimen of 1 g of tumour tissue was minced with scissors in Hanks' balanced salt solution (HBSS) and homogenized in 10 ml HBSS with a glass homogenizer at 4°C. The homogenate was centrifuged at 5,000 *g* for 10 min and the supernatant was dialysed through a Spectra/Por 3 membrane (Visking Co.) (exclusion molecular weight $>3{,}500$) against 20 volumes of water. The dialysate and the residual material were tested for eosinophil chemotactic activity after serial dilution with RPMI 1640 (Gibco, Grand Island NY, U.S.A.).

Measurement of chemotaxis

A modification of the Boyden micropore filter technique[9] was used. Guinea pig eosinophils were obtained by peritoneal lavage from animals which had received weekly injections of 2 ml horse serum (Nakarai Chemicals Ltd., Kyoto, Japan) for 8 to 12 weeks.[10] On the 5th day after the last injection, peritoneal cells containing more than 65% eosinophils were collected in HBSS containing 10 units/ml sodium heparin (Nakarai Chemicals), and washed and resuspended in RPMI 1640 medium containing 0·5% 5-times crystallized ovalbumin (Sigma Chemical Co., St. Louis, MO, U.S.A.) at a concentration of 5×10^5 cells/ml. The upper compartment of each blind-well chamber (Nuclepore Corp., Pleasanton, CA, U.S.A.) contained 0·75 ml of the eosinophil suspension. The lower compartment was filled with 0·2 ml of the tissue extract diluted in RPMI 1640 medium. Guinea pig sera activated with 2 mg/ml Zymosan A (Sigma) and RPMI 1640 medium were used as positive and negative controls respectively.

A polycarbonate filter with 5·0 μm pores (Nuclepore) separated the two compartments. After incubation for 90 min at 37°C in 5% CO_2 95% air the filters were removed, fixed in $HgCl_2$-ethanol solution for 15 h and stained with Carrazzi's haematoxylin and Chromatrope 2R (Schmid GMBH & Co., Stuttgart-Untertürkheim).[11] The number of eosinophils migrating into the filters was counted in 10 randomly selected high power fields (hpf) at × 40 objective magnification. The chemotactic activity was taken as the mean number of cells/hpf in duplicate filters for each sample.

Gel filtration

The tissue extract was fractionated by gel filtration on a Sephadex G-50 column (1·5 × 46 cm) (Pharmacia). One ml of dialysed extract was added to the column and 0·067 M phosphate buffered saline was used as the eluent. Blue dextran, cytochrome c and vitamin B_{12} were used as molecular weight standards. Each fraction was diluted 1 : 10 in RPMI 1640 and 0·1 ml volumes used for measurement of chemotactic activity as described above.

RESULTS

The eosinophil chemotactic activity of the tissue extract is shown in Figure 1. The tissue dialysate demonstrated chemotactic activity in a dose-dependent fashion, but residual material showed no chemotactic activity. Figure 2 shows the chemotactic activity of the Sephadex G-50 fractions of the tissue dialysate. The peak chemotactic activity was eluted in the fraction of molecular weight approximately 1000 daltons.

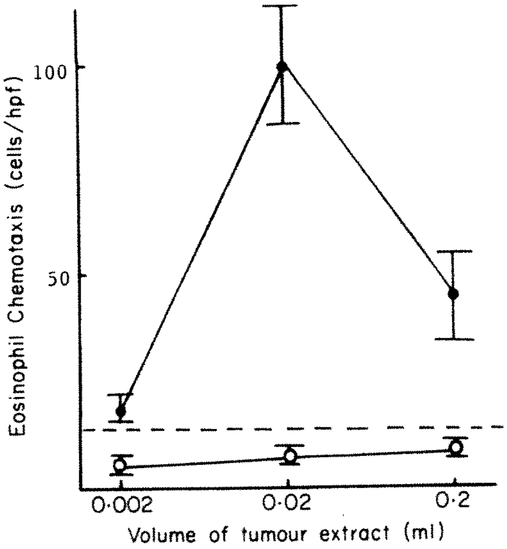

FIGURE 1. Eosinophil chemotactic activity of tumour extract from a patient with Kimura's disease. ●——●: dialysate; O——O: residual material. Points are means ± SD. Spontaneous eosinophil motility (control) gave a mean of 12·7 cells/hpf (- - - -).

DISCUSSION

In the present study we have detected eosinophil chemotactic activity in an extract of tumour tissue from a patient with Kimura's disease. After Sephadex gel filtration, the activity was obtained in the fraction of molecular weight approximately 1000 daltons. A low molecular weight eosinophil chemotactic factor has previously been demonstrated in several types of white blood cells,[12–15] in the granulomatous liver of animals infected with *Schistosoma mansoni*[16] and in blister fluid from patients with contact dermatitis and atopic eczema.[17] The chemotactic factors from all these sources had molecular weights between 300 and 500 daltons.

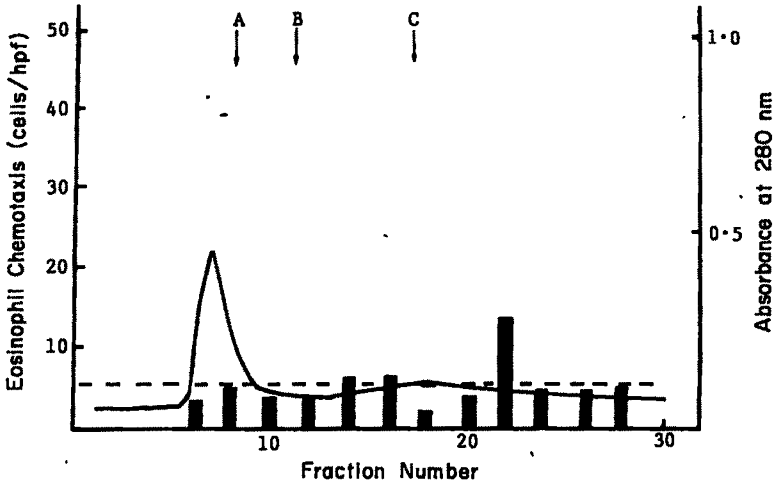

FIGURE 2. Eosinophil chemotactic activity of Sephadex G-50 fractions of tissue dialysate (bars), and absorbance at 280 nm (———). Molecular weight standards: A, blue dextran (mol. wt. 2×10^6 daltons); B cytochrome c (mol. wt. 15,000 daltons) and C, vitamin B_{12} (mol. wt. 1355 daltons). Spontaneous eosinophil motility (control) gave a mean of 5·5 cells/hpf (- - - -).

Although the aetiology of Kimura's disease is not still understood, an atopic allergic origin has been suggested on the basis of eosinophilia, elevated serum IgE levels and appearance of IgE antibodies against *Candida albicans*, and localization of IgE in the germinal centres of lymphoid follicles.[8] These results suggest the possibility that mast cells may be responsible for the production of this substance, as is the case with the eosinophil chemotactic factor in anaphylaxis, because of IgE-mediated immune reactions. Kay *et al.*[18] also reported that a low molecular weight eosinophil chemotactic factor was present in supernatants from tissue culture of lymph node cells from a patient with Hodgkin's disease.

The molecular events and the cell types responsible for production or secretion of the eosinophil chemotactic factor were not determined in the present study. However, it appears that this factor may play a specific role in causing the selective accumulation of eosinophils in Kimura's disease.

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Expression of a pemphigoid gestationis-related antigen by human placenta

SUSAN E.KELLY, B.S.BHOGAL*, FENELLA WOJNAROWSKA
AND M.M.BLACK*

Departments of Dermatology, The Slade Hospital, Headington, Oxford and *Institute of Dermatology, St John's Hospital for Diseases of the Skin, London, U.K.

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SUMMARY

We studied the antigen binding of sera from 25 patients with pemphigoid gestationis (PG) using skin and amnion as substrates in an indirect immunofluorescence technique. In addition, skin was studied after prior incubation with 1 M NaCl which allows BMZ cleavage through the lamina lucida.

Linear BMZ staining was obtained with sera from patients with PG using human amnion as substrate. This was seen with both full-term and second trimester placentae, but not with first trimester placentae. In NaCl incubated skin all sera bound to the epidermal aspect of the separated tissue. The pattern of staining in skin, chemically split skin and amnion was identical to that previously seen with the sera of patients with bullous pemphigoid suggesting that the antigen against which circulating anti-BMZ antigens are directed may be the same in the two conditions.

Pemphigoid gestationis (PG) is a rare blistering disease of pregnancy characterized by the development of subepidermal bullae and liquefaction degeneration of the epidermal basal layer.[1] It is most frequently associated with pregnancy, but there are reports of PG in association with hydatidiform mole[2,3] and choriocarcinoma.[4]

There appear to be similarities between PG and bullous pemphigoid (BP). In both diseases subepidermal bullae develop which are thought to be caused by immune mediated tissue damage. Deposits of immunoglobulin and C_3 may be detected in the basement membrane zone (BMZ) of perilesional skin in both diseases. Circulating anti-BMZ antibodies may be detected in the serum of both groups of patients, and in BP such antibodies have been shown to be pathogenetic in experimental animal models of the disease.[5]

Circulating auto-antibodies are, however, less frequently detected in the sera of patients with PG than with BP. When present, the auto-antibody in PG has been shown to be complement

Correspondence: Dr S.E.Kelly, Department of Dermatology, Royal Berkshire Hospital, London Road, Reading, Berkshire RG1, U.K.

fixing[6] and even in cases in which there is no detectable circulating anti-BMZ antibody, a serum anti-BMZ complement fixing agent may be detected.[7] This poorly defined molecule is termed PG factor. When anti-BMZ antibodies are detected they are most frequently of IgG class[8] and PG factor is almost certainly an IgG antibody. The antigen against which PG factor is directed has not been characterized. In addition to the obvious clinical relationship between PG and pregnancy there is evidence of placental insufficiency in some patients with PG.[9] This relationship has suggested that the antigen may be a molecule present both in the placenta and in the skin. Cross reactivity has been identified between several types of antigens in the amnion and in the skin.[10] This cross reactivity may not be surprising since skin and amnion are both derived from the ectodermal germ layer during embryogenesis.[11] In addition, abnormal expression of HLA-DR antigen has been identified in the capillary endothelium of some chorionic villi from the placenta of a patient with PG.[12] We have studied the antigen binding specificity of PG serum using an indirect immunofluorescence (IF) technique and have tested cross reactivity between the skin and placenta.

METHODS

Tissue

Full term placentae were collected from five normal pregnancies during vaginal delivery. Early placentae were obtained during five legal abortions at 12 weeks gestation and from five spontaneous abortions at 22 weeks. Gestational age was estimated by menstrual dates and confirmed by measurement of foetal crown–rump length. Amnion placentae (amniochorionic membrane) and amnion from umbilical cord were studied individually. Skin specimens from 10 healthy adult volunteers with no evidence of skin disease, were obtained during minor surgical procedures. All tissue was snap frozen in liquid nitrogen at −70°C and stored at −70°C. Samples of these tissues were stained with haematoxylin and eosin and were found to be morphologically normal. Appropriate tissue blocks were mounted in OCT (Ames Co) and a 6 μm cryostat section was cut and stained with toluidine blue to confirm the orientation of the specimen. Multiple adjacent sections were cut and stained by immunofluorescence techniques.

Normal human skin was chemically separated by incubation with 1 M NaCl as described by Scaletta.[13] Briefly, strips of skin were incubated in 1 M NaCl for 48–72 h until the epidermis was easily removed from the dermis by gentle traction. Thus separated, the skin was snap frozen in liquid nitrogen at −70°C and stored at −70°C. We have previously shown that skin which has been incubated in 1 M NaCl and separated through the lamina lucida provides a sensitive assay for circulating anti-BMZ antibodies in BP.[14]

Serum

Serum was collected from 25 patients with PG. PG had been diagnosed on the basis of typical clinical and histological findings and confirmed on direct immunofluorescence by demonstration of a linear band of IgG or C_3 or both in the cutaneous BMZ of perilesional skin. The sera were stored at −20°C.

Indirect immunofluorescence

Standard IF techniques were used.[15] Briefly, 6 μm cryostat sections were cut from frozen blocks of skin and amnion. These were air dried for 15 min and rinsed in phosphate buffered saline (PBS) at pH 7·4 for 15 min. Sections were then incubated at 37°C for 30 min with patient's serum diluted 1 : 10 in PBS, washed with PBS for 15 min and then incubated at 37°C for 30 min with FITC labelled rabbit anti-human IgG (Dako). After a final wash in PBS, sections were mounted in buffered glycerol and examined by ultraviolet microscopy. The indirect C_3 method as described by Hodge *et al.*[16] was employed. Sections were sequentially incubated with (i) patient's serum diluted 1:4 with PBS, (ii) normal human serum diluted 1:5 with complement diluting buffer (a source of complement), (iii) FITC labelled rabbit anti-human C_3. All incubations were for 30 min at 37°C and sections were washed for 15 min in PBS between incubations. Ten normal sera from volunteers with no evidence of skin disease replaced test serum for control experiments.

RESULTS

Results of IF using amnion as substrate are given in Table 1 and are compared with results using intact skin and skin incubated in 1 M NaCl and separated through the lamina lucida. A positive result was defined as a brightly fluorescent linear BMZ band. Weak staining of the basal cytoplasm of some amniocytes was seen in addition to the basement membrane staining.

TABLE 1. Indirect immunofluorescence using sera from 25 patients with pemphigoid gestationis on different substrates

	No. of sera positive			
	Normal skin	1 M NaCl split skin	Amnion placentae	Amnion cord
C_3	24	23	22	22
IgG	15	20	22	15

IgG

Sera from 15 of 25 patients were found to contain circulating IgG anti-BMZ antibody when tested on intact skin. Use of separated skin as substrate increased the detection rate to 20 of 25. All sera bound to the epidermal side of the lamina lucida. In 22 of 25 sera there was circulating IgG bound to the BMZ of placental amnion (Fig. 1) and in 15 of 25 circulating IgG bound to cord amnion.

C_3

Twenty four of 25 sera were found to contain a circulating complement fixing BMZ factor when tested on intact skin. When chemically split skin was used 23 of 25 sera were positive and all bound to the epidermal side of the lamina lucida. In 22 of 25 sera this factor bound to the amniotic BMZ both in placenta (Fig. 2a) and umbilical cord (Fig. 3).

Ontogeny

Nine sera which were positive for C_3 and IgG on full-term placentae were tested against early placental tissue. All nine sera were positive for both complement fixation and circulating IgG on amnion placentae from 22 weeks gestation placentae and all were negative on amnion placentae from 12 weeks gestation placentae. Amnion cord from these placentae was not available.

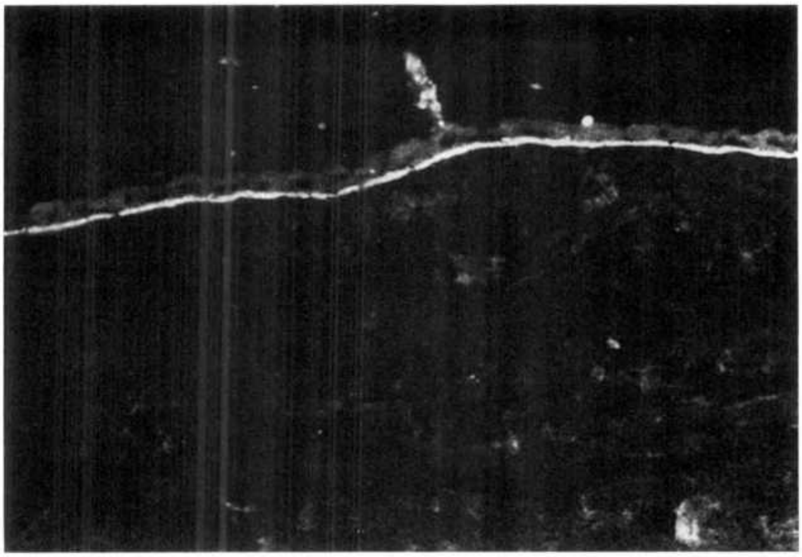

FIGURE 1. Linear fluorescence in the amnion BMZ in indirect IgG immunofluorescence confirms that the anti-BMZ antibody is IgG. (Original × 250).

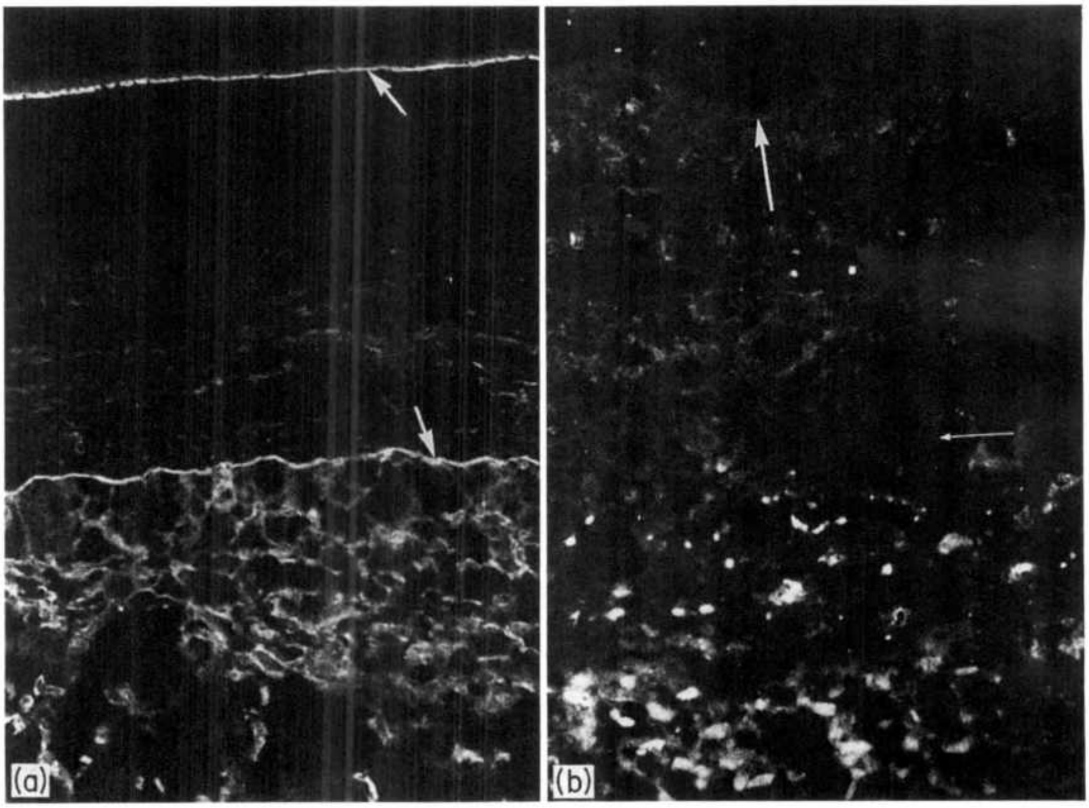

FIGURE 2(a). Reactivity of PG serum on placental amnion. Indirect C_3 immunofluorescence. There is linear fluorescence in the amnion basement membrane zone and in the chorionic plate (arrows) (original × 160). (b) Control serum on placental amnion shows only diffuse non specific staining. Arrows indicate BMZ (original × 160).

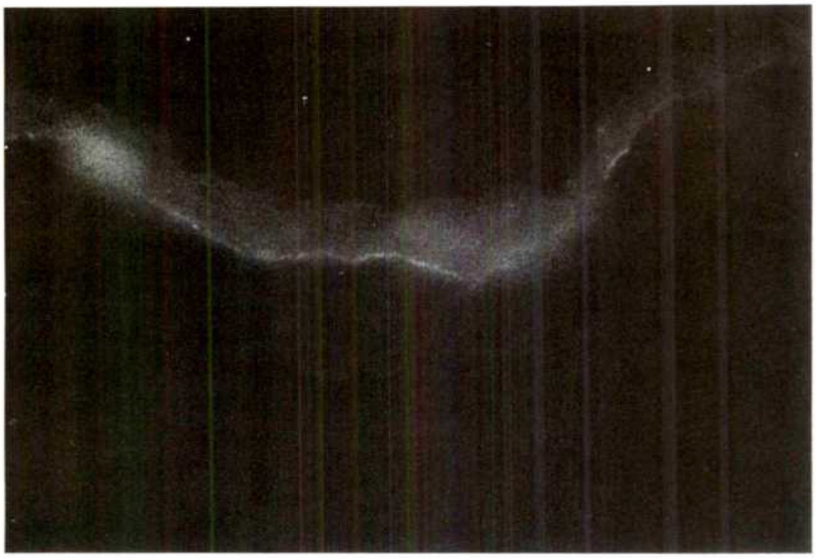

FIGURE 3. Linear BMZ fluorescence in the cord amnion using the indirect C_3 technique. (Original × 1000).

Controls

All controls were negative on all substrates (Fig 2b).

DISCUSSION

Pemphigoid gestationis is considered to have an autoimmune aetiology. The obvious association between PG and pregnancy suggests that immune mediated tissue damage follows exposure to a newly encountered antigen produced or exposed during gestation.

In BP, circulating antibodies are known to be produced and directed against the bullous pemphigoid antigen (BPA), although other as yet uncharacterized constituents of the BMZ may also be of importance in the pathogenesis of BP. BPA is a glycopeptide macromolecule produced by basal keratinocytes and is a constituent of normal squamous epithelium. This glycoconjugate is found in association with hemidesmosomes in the basal keratinocytes and there exists a large intracellular pool of BPA.[17] Immunoprecipitation studies have identified this molecule as a 220 kd protein.[18] Although this molecule has not been extracted from placentae, IF studies with BP sera produce linear BMZ staining in amniotic membrane[19] confirming that placental tissue contains an antigenic determinant recognized by BP serum. This may be similar or identical to BPA. Further evidence that antigens shared by skin and amnion may be involved in pathological reactions is suggested by a report of a bullous pemphigoid-like dermatosis occurring in a patient after he had received therapeutic injections of placental extract.[20]

The antigen in PG awaits full identification and characterization. The relationship to pregnancy suggests that the antigen may be a molecule present both in placenta and in skin. Verrando *et al.*[10] have identified a shared antigen present in amnion and skin, biochemical analysis of which suggests that it is a 37 kd protein. This group has also demonstrated anti-placental antibodies in the sera of a small group of patients with PG.[21] This was measured in terms of complement deposition and was identified in the amnion and in the syncytiotrophoblast.

We chose to study amnion placenta and amnion from umbilical cord individually, as histologically amnion placenta and amnion cord differ in cellular morphology. The placental amnion is composed of a single layer of columnar cells while the cord amnion is a statified epithelium.[22]

Our study shows that PG sera identify an antigen present in the BMZ of amnion placentae and amnion cord. Sera also produced linear staining in the chorionic plate BMZ, but we were unable to demonstrate any specific staining in chorionic villous stroma. Weak staining of the basal cytoplasm of some amniocytes was seen in addition to the basement membrane staining. Although this was not demonstrated with control sera, it is difficult to determine whether this represents true intracellular staining or is a reflection of the orientation of the tissue specimen. Unlike the results reported by Ortonne,[21] the sera from our patients did not show specific staining of cytotrophoblast or syncytiotrophoblast cytoplasm. The antigen which we have identified was present from 22 weeks gestation until term, but was absent from 12 weeks gestation placentae. This suggests that the antigen develops in the amnion during the second trimester of pregnancy. The time of onset of PG in any pregnancy is variable, but most often occurs in the second or third trimester. Occasionally the cutaneous eruption may start as early as 9 weeks.[9] However, in first affected pregnancies the condition tends to commence at a later gestational age and it may be that prior exposure to the placental antigen in the second trimester of the first pregnancy is required for the disease to manifest itself.

Incubation of human skin with 1 M NaCl is known to chemically separate skin through the lamina lucida leaving BPA in close association with the epidermis.[23] Using chemically split skin as substrate for IF, the antigen recognized by PG sera was localized to the epidermal aspect of the lamina lucida. This is the pattern most commonly identified in BP. Previous ultrastructural studies in PG have localized immunoreagent deposition to the lamina lucida with a marked uniformity throughout. In PG there appears to have been no accentuation at the site of hemidesmosomes.[24]

It is still unclear what is the primary event in the pathogenesis of BP and PG. Clearly, in normal skin the molecule against which antibodies are directed is not recognized as antigenic. In addition, the serum titre of circulating antibodies does not vary with disease activity and it is considered that antibody production may be a secondary phenomenon. The formation of bullae which alter the structure of the BMZ may expose epitopes in antigenic molecules or induce conformational changes in BMZ constituents. Once antibodies are synthesized, however, they are clearly responsible for perpetuating the disease, due in part to complement activation and cell lysis. Further *in vitro* models of disease activity require study to elucidate this.

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Acanthosis nigricans, insulin resistance and cutaneous virilism

J.H.BARTH, L.L.NG*, F.WOJNAROWSKA AND R.P.R.DAWBER

Department of Dermatology, The Slade Hospital, Oxford, *Sheikh Rashid Diabetes Unit, Radcliffe Infirmary, Oxford, U.K.

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SUMMARY

Thirteen patients with the syndrome of acanthosis nigricans and insulin resistance are described. They all had a combination of dermatoses related to hyperandrogenism (cutaneous virilism): hirsuties (11), acne vulgaris (6), hidradenitis suppurativa (5) and androgenic alopecia (4). In addition, 9 out of 13 had keratosis pilaris. The patients had raised fasting plasma insulin levels compared with matched normal controls ($P<0.01$) and increased insulin resistance ($P<0.02$). Insulin resistance correlated with total serum testosterone ($r_s=0.65$; $P<0.02$).

Acanthosis nigricans (AN) and insulin resistance are features of many congenital, metabolic and endocrine disorders, for example, Cushing's disease, acromegaly, leprechaunism, Laurence-Moon-Bardet-Biedel and Prader-Willi syndromes and congenital lipodystrophy.

A relationship between diabetes and hyperandrogenism in women or diabetes of bearded women has been recognized for many years.[1,2] However, it is only in the past few years that the cause of the disordered carbohydrate metabolism has been defined as resistance to insulin;[3] and that the combination of AN and insulin resistance (IR) is found in as many as 5% of women with hyperandrogenism (HA).[4] The acronym HAIR-AN has been used to describe this syndrome; even though, as we found in the present study, not all the women are hirsute.

We have studied hyperandrogenized women, most of whom have presented to a dermatology department with hirsuties, in order to determine the features of cutaneous virilism associated with AN and insulin resistance and the relationship of the latter with serum androgens.

METHODS

Patients

Two hundred and twenty seven patients were referred for assessment of hirsuties. Seventy four (33%) were both sufficiently hirsute (Ferriman and Gallwey[5] grade > 15) and adequately motivated to undergo prolonged anti-androgen therapy. Sixteen (22%) of the 74 patients had

Correspondence: Dr J.H.Barth, Department of Dermatology, The Slade Hospital, Oxford OX3 7JH, U.K.

acanthosis nigricans and nine (12%) were studied for insulin resistance. Four further hyperandrogenized women with acanthosis nigricans (two with hidradenitis suppurativa and two with generalized keratosis pilaris) were also studied. The study had the approval of the Central Oxford Research Ethical Committee.

Grading schemes

Hirsuties was measured by the Ferriman and Gallwey scale;[5] and androgenic alopecia by Ludwig grades.[6] Obesity was measured as the body mass index (BMI)[7] calculated as weight/height^2 in kg/m^2. The diagnosis of polycystic ovary syndrome was based on menstrual cycle lengths >35 days and non-midcycle luteinizing hormone >10 U/l.

Laboratory methods

Sex hormone binding globulin (SHBG) was measured by the method of Iqbal and Johnson.[8] Insulin was assayed by radio-immunoassay;[9] the coefficient of variation for this assay is 6% and the sensitivity 2 mU/l.

The insulin resistance for each subject was determined from the fasting plasma insulin and glucose levels using a computer solved model [homeostasis model assessment (HOMA)]. This model calculates the insulin resistance in the fasting state which is assumed to be a steady-state where glucose production equals glucose disposal. HOMA uses the best available physiological data for the effects of glucose and insulin on hepatic glucose production, glucose uptake by muscle, adipose and nervous tissue. A near approximation for calculating insulin resistance is:

$$I/22{\cdot}5 \times e^{-\ln G}$$

where I is fasting insulin and G is fasting glucose. A healthy individual of normal weight is arbitrarily allocated an insulin resistance value of 1·0. The insulin resistance thus calculated correlates well with that derived from the hyperinsulinaemic euglycaemic clamp.[10]

Statistics

The Oxstat statistical package was used. Mann Whitney U tests and Spearman correlation coefficients for non-parametric data were used. Median values and ranges are quoted and P values are two-tailed.

RESULTS

Clinical features

The clinical details are summarized in Table 1.

Acanthosis nigricans. AN was recorded in 7% of the 227 cases (although its presence was not recorded for 153 of these cases who were not studied). The AN was identical in appearance to the form associated with malignant disease. There were verrucous plaques which varied from soft light brown velvety areas to thickened dark brown rough plaques with deep pseudo-fissures (Fig. 1). They principally affected a thin necklace-like band on the lateral and dorsal aspects of the neck; the majority of the patients also had AN of the axillae and inner thighs. The more obese subjects had AN in the intertriginous areas under the breasts and in the fatty fold around the waist and in two cases the skin overlying the sternum was affected. There were pigmented verrucous plaques over the elbow tips in two cases and on the dorsal aspects of the interphalangeal joints in a further case; these were all considered to be examples of AN. The palms were not involved in any patient.

TABLE 1. Clinical details and laboratory findings in 13 patients with HAIR-AN syndrome

Patient no.	Age years	BMI*	Total testosterone nmol/l	SHBG ng DHT bound/l	Insulin resistance HOMA (arbitrary units)	PCOS‡	Acne vulgaris**	Hirsuties†	Androgenic alopecia Ludwig[6] grade	Hidradenitis suppurativa	Extensive keratosis pilaris
1	24	24·2	2·5	22·9	3·63	−	+	17	...	+	...
2	34	24·3	1·8	43·5	8·03	−	...	0	II	...	++
3	35	28·4	2·8	7·0	5·35	+	+	27	II	...	+
4	31	31·6	1·7	49·5	5·00	−	...	36	II	...	...
5	35	34·1	2·5	13·2	1·71	+	...	35	...	...	...
6	34	34·2	2·7	62·9	4·45	−	...	31	...	...	+
7	37	34·6	3·9	20·8	12·04	+	++	34	...	+	...
8	16	34·6	5·2	7·6	17·16	+	+	37	...	...	++
9	22	35·6	2·1	10·1	2·55	−	++	37	II	+	++
10	36	40·1	7·8	26·8	18·30	+	...	36	...	...	++
11	36	41·3	1·1	33·0	8·28	−	...	0	...	...	++
12	39	44·4	8·8	38·5	15·20	+	...	27	...	+	+
13	35	46·8	3·4	N/A	8·36	+	+	17	...	+	+

* BMI = Quetelet Index, weight/height^2

** Acne: + mild ++ moderate +++ severe

† Hirsuties, Ferriman and Gallwey scale[5] (maximum score 44)

‡ PCOS: Polycystic ovary syndrome

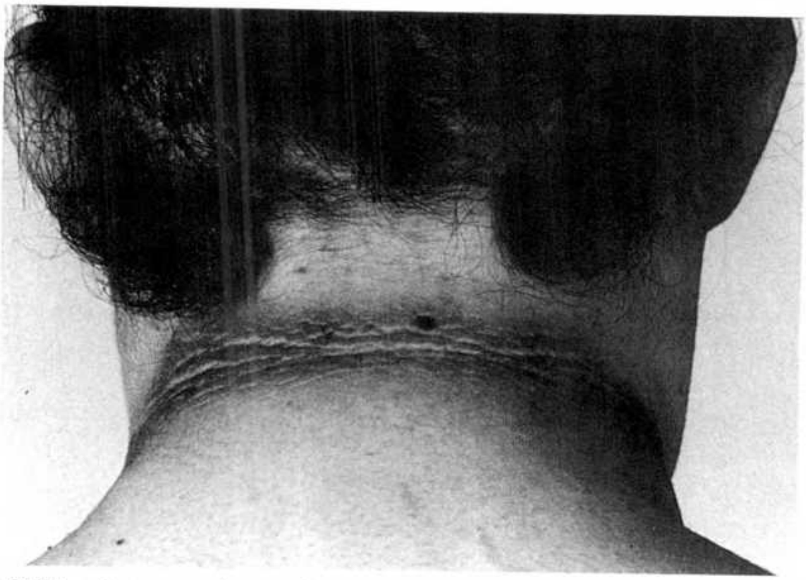

FIGURE 1. Thickened verrucous pigmented 'necklace' of acanthosis nigricans with deep 'pseudo-fissures' on the back of the neck of a 16-year-old obese hirsute woman (Patient 8).

Obesity. The median BMI was 34·6 (range 24·2–46·8), which indicated that the group was considerably overweight (BMI 25·8 is approximately 120% and BMI 30 is approximately 140% of ideal body weight).

The other features of cutaneous virilism are summarized in Table 1. The median hirsuties grade 34 (range 17–37)—excluding the two non-hirsute women—is artificially high as the source of referral produced an inevitable bias towards severe hirsutism.

Metabolic data

The thirteen hirsute women with AN were compared with 13 controls matched for age (median age, patients 35 years, range 16–39; controls 40 years, range 20–57) and BMI (mean patients 34·6, range 24·2–46·8; controls 32·2, range 21·7–57·6, difference not significant). Median fasting insulin was 33 mU/l (range 7·7–65·0 mU/l) in the patients and 10·4 mU/l (range 1·5–33·3 mU/l) in the controls ($P < 0·01$). Median fasting glucose was 5·1 m mol/l (range 4·4–8·0) for patients and 5·1 mmol/l (range 4·4–6·9 mmol/l) in the controls; this difference was not significant. Median insulin resistance (HOMA) in arbitrary units was 8·03 (range 1·71–17·16) in the patients and 2·44 (range 0·33–8·62) in the controls ($P < 0·02$) (Fig. 2).

The control group were slightly, although not significantly, older. This would have the effect of reducing the difference demonstrated, as insulin resistance increases with age.[11]

Endocrine data

Median serum testosterone was 2·7 nmol/l (range 1·1–8·8); SHBG 22·9 ng DHT bound/l (range 7·0–62·9). Testosterone levels correlated with insulin resistance, $r_s = 0·65$ ($P < 0·02$) (Fig. 3), but not with BMI, $r_s = 0·46$ ($P = 0·12$).

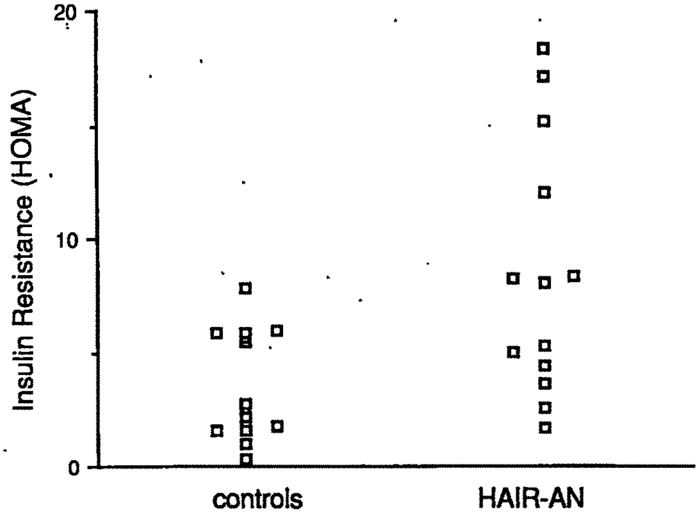

FIGURE 2. Insulin resistance (HOMA) in arbitrary units in patients with HAIR-AN syndrome and in age and weight matched controls.

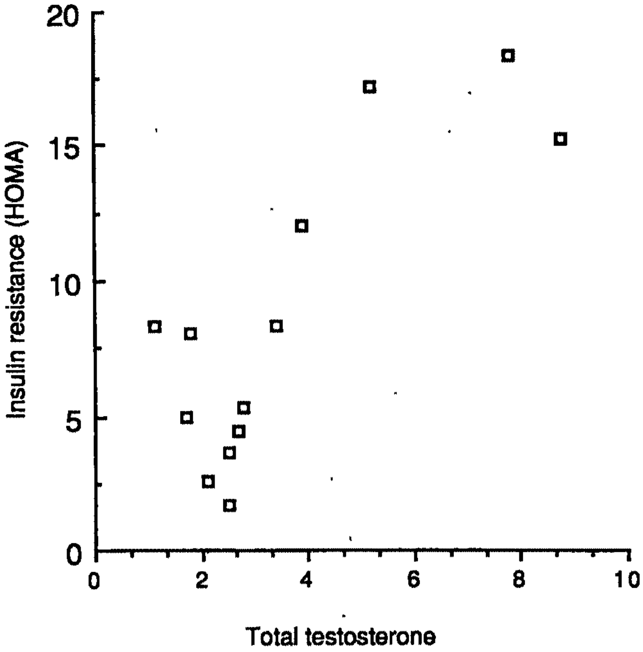

FIGURE 3. The relationship between insulin resistance (HOMA) in arbitrary units and the total serum testosterone in patients with HAIR-AN syndrome. $r_s = 0.65$ ($P < 0.02$).

DISCUSSION

We have studied patients with acanthosis nigricans (AN) who have presented with features of cutaneous virilism. In the group of hirsute patients the incidence of AN and insulin resistance was at least 7% (16 of 227) as this figure represents the number of patients in whom AN was recorded. This figure may be falsely low as only a third of the total group of patients was studied; however, the incidence of 7% is similar to that in the report by Flier *et al.*[4] which suggested an incidence of 5%, but did not include a definition of AN.

The features of cutaneous virilism are quite pronounced and reflect the degree of hyperandrogenism which has been demonstrated to be directly proportional to insulin resistance. An unusual aspect of this series is that only half the patients had polycystic ovary syndrome (as defined by oligomenorrhoea and increased luteinizing hormone levels) whereas this is usually considered to be a prerequisite feature.[4,12–15]

The pathophysiological mechanism associating hyperandrogenism, insulin resistance and acanthosis nigricans is not known. Neither is it known whether hyperandrogenism precedes the insulin resistance. There is clearly a close relationship between the level of serum testosterone and insulin resistance as demonstrated in the present and previous reports.[12,13] This relationship is not dependent upon obesity, as shown in two of our cases and one previous report.[13]

The development of acanthosis nigricans at puberty with the normal surge of androgen production would suggest that androgens have an aetiological effect.[16–19] It is not known whether androgens act directly on the epidermis to produce AN or indirectly by the induction of a state of insulin resistance.[20] There are two possible mechanisms for an insulin mediated effect; insulin may directly stimulate epidermal transformation or it may enhance ovarian androgen production. Both these effects may be created by the binding of insulin to receptors for insulin-like growth factors in the epidermis or ovary. Increased ovarian production of androgens would aggravate the condition with the formation of a positive feedback mechanism.

Treatment of acanthosis nigricans with anti-androgenic agents has been unsuccessful in our experience (five of the patients in the present series received 12 months of anti-androgen therapy with no remission of their acanthosis nigricans). However, there have been isolated case reports of temporary remissions induced by other therapies. In one report, surgical ovarian wedge resection was successful,[21] in another, therapy with cyclical norethindrone and mestranol in conjunction with ovarian wedge resection was effective,[19] and in a third, drug therapy alone with cyproterone acetate was successful at reducing AN.[22] Weight reduction may be effective in reducing insulin resistance,[15] but has only been reported to be effective in reducing acanthosis nigricans in a single case.[4]

ACKNOWLEDGMENTS

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Combined cold urticaria and cholinergic urticaria—clinical characterization and laboratory findings

A.D.ORMEROD, A.KOBZA-BLACK, A.MILFORD-WARD*, AND M.W.GREAVES

St. John's Hospital for Diseases of the Skin, Lisle Street, London, and *Protein Reference Laboratory, Royal Hallamshire Hospital Medical School, Sheffield, U.K.

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SUMMARY

Thirteen patients with both cold and cholinergic urticaria are reported. There was considerable variability, particularly in the cold urticaria which was of the common cold contact type in seven patients, of the generalized cold induced cholinergic type in two and in four patients the lesions induced by direct contact with ice were morphologically like cholinergic urticaria, but appeared despite prior application of an acetyl choline antagonist. The natural history, laboratory findings and the effects of therapy are discussed.

The occurrence of more than one physical urticaria in the same patient has long been recognized and there are several isolated reports of cold and cholinergic urticaria occurring together. The first report of a patient reacting to cold, exercise and warming of the body was published in 1936.[1] Two years later four similar patients with a generalized reaction to heat and a local reaction to cold were reported.[2] Since these initial reports 14 other cases of cold and cholinergic urticaria have been reported.[3–12] More recently the release of histamine into the circulation following both cold challenge and exercise has been reported in these patients,[8–10] and in the extensive series of 220 patients with cold urticaria reported by Neittaanmaki,[13] 17 also had cholinergic urticaria.

In some patients with a history suggestive of cold or cholinergic urticaria, exercise tests, hot showers and ice cube tests produce no reaction, but cooling the body in a cold room at 4°C for 10–20 min leads to a generalized urticaria which is visually indistinguishable from cholinergic urticaria.[14] Recently this type of generalized cold induced cholinergic-like urticaria was described in association with common heat induced cholinergic urticaria.[11]

Correspondence: Dr A.D.Ormerod, Ward 29, Dermatology, Aberdeen Royal Infirmary, Foresterhill, Aberdeen AB9 2ZB, U.K.

In the last 7 years we have seen 13 patients with both cold and cholinergic urticaria and we present here a review of their clinical features and laboratory findings.

METHODS

For the purpose of the present study we defined cholinergic urticaria as urticaria occurring following the induction of sweating by a hot bath or by exercise on a stationary bicycle. Cold urticaria was defined as a wealing response to the application of ice to the skin for 10 min (cold contact urticaria) or an urticarial reaction following cooling in a cold room at 4°C with loose clothing for 10–20 min. All patients studied had reactions both to cold and to stimulation of sweating.

All 13 patients were seen during a 7-year period by one of the authors. Nine also responded to a detailed questionnaire regarding precipitating factors, associated symptoms, effects of treatment and the course of each type of urticaria. Of these patients seven were re-examined at the time of the present study. The effects of each treatment used on both kinds of urticaria were assessed retrospectively from case records and questionnaire responses as helpful or unhelpful.

Laboratory investigations carried out included the following: full blood count (13 patients), urea, electrolytes and liver function tests (9), antinuclear factor (9), immunoglobulins IgG, IgA, IgM and IgE (8), C_3 and C_4 levels (10), C_1 esterase inhibitor (8), cryoglobulins (11), cold

TABLE 1. Clinical details of patients with cold and cholinergic urticaria

	Cold urticaria				Cholinergic urticaria		
Patient no.	Duration (years)	Type of urticaria*	Severity	Systemic symptoms	Duration (years)	Severity	Systemic symptoms
1	9	A	severe	severe	10	severe	severe
2	21	A	mild	—	21	severe	moderate
3	7	A	moderate	—	6	moderate	mild
4	5·5	A	moderate	—	6	moderate	—
5	2‡‡	B	moderate	—	11	severe	severe
6	5‡‡	B	severe	mild	5	mild	—
7	10	C	moderate	—	10	severe	—
8	?‡‡	C	incidental	—	17	severe	moderate
9	3**	C	mild	—	1	moderate	—
10	10†	C	severe	—	11	severe	—
11	26‡	C	severe	moderate	26	mild	—
12	4	C	moderate	—	?	incidental	—
13	2‡‡	C	severe	—	6	severe	mild

*A = Cholinergic-like cold contact
B = Generalized cold induced cholinergic-like
C = Classical (confluent) cold contact
** Iron deficiency
† Rheumatoid factor 1:32
‡ Rheumatoid factor 1:250
‡‡ Also had dermographism

agglutinins (11), cryofibrinogen (3), *Treponema pallidum* haemagglutination test (7), venereal disease research laboratory test (7), hepatitis B surface antigen (6), *Mycoplasma pneumoniae* complement fixation test (7), rheumatoid factor (5) and protease inhibitors (6). Protease inhibitors were measured as previously described.[15]

RESULTS

The clinical features of the patients are summarized in Table 1. There were 10 females and three males, age range 11–53 years (mean 28 years). All patients exhibited classical cholinergic urticaria in response to sweat provoking stimuli, but patterns of cold urticaria were varied. Seven patients had a confluent reaction at the site of cooling and a history of large weals termed 'confluent cold contact urticaria'. Four patients had a morphologically distinct local reaction to ice, closely resembling cholinergic urticaria (Fig. 1) and termed 'cholinergic-like cold contact urticaria'. Unlike true cholinergic urticaria this could not be prevented by prior topical application of sufficient 10% aqueous hyoscine to prevent sweating at the site. Two patients had negative ice cube tests, but when the whole body was cooled in the cold room developed a generalized rash identical to that of cholinergic urticaria as previously described[13] and termed 'generalized cold induced cholinergic-like urticaria'.

Four patients also demonstrated dermographism on testing with a spring-loaded dermographometer as described by Breathnach *et al.*;[16] this was symptomatic in two patients. During severe attacks eight patients noted systemic symptoms including dizziness, headache, dyspnoea, nausea, fainting, diarrhoea and palpitations; the types of symptoms were similar in both cold and cholinergic episodes.

All laboratory investigations were normal except for a coincidental iron deficiency anaemia in one patient and positive rheumatoid factors (sheep cell agglutination test) at titres of 1:32 and 1:250, respectively, in the sera of two patients. C_1 esterase inhibitor was minimally raised in the sera of four patients and was normal in four (Fig. 2). The results of plasma protease inhibitor

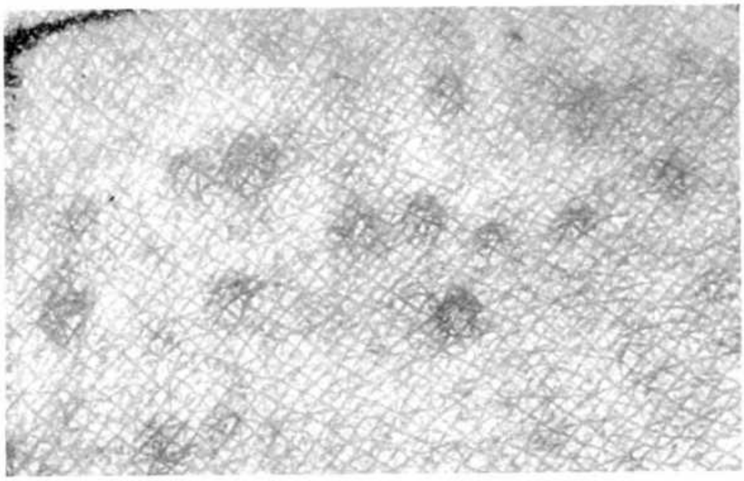

FIGURE 1. Cholinergic-like cold contact urticaria.

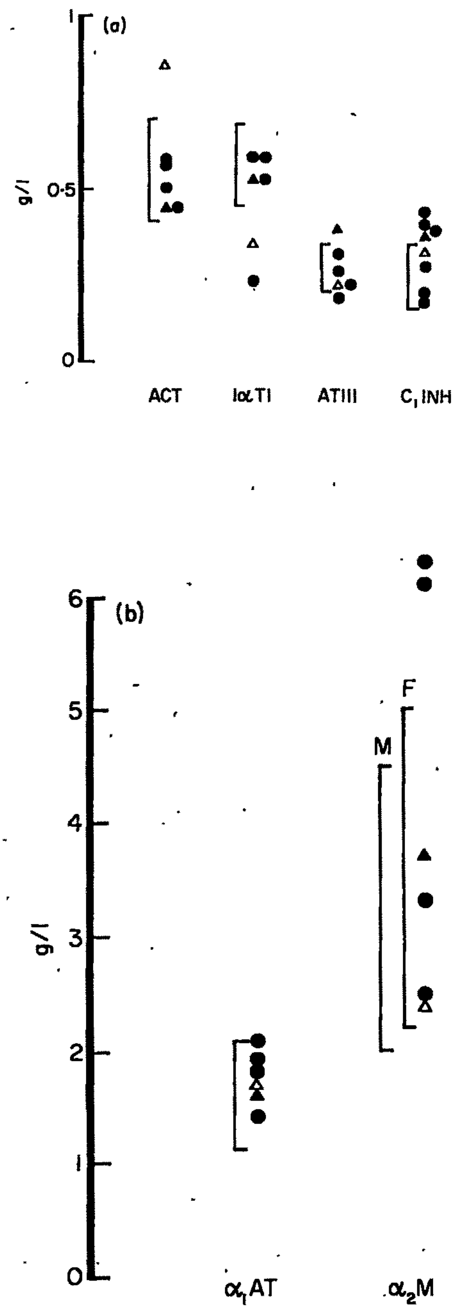

FIGURE 2. Protease inhibitor profiles in cold and cholinergic urticaria patients compared with the reference range (mean ± SD) for the normal population. (a) α_1-antichymotrypsin (ACT); inter-α-trypsin inhibitor (IαTI); antithrombin III (ATIII) and C_1 esterase inhibitor (C_1INH). (b) α_1-antitrypsin (α_1AT) and α_2 macroglobulin (α_2M) (normal ranges for males (M) and females (F) indicated separately). △ = Male in whom urticarias had resolved one year previously. ▲ = Male with active urticaria. ● = Female with active urticaria. Vertical bars = Normal range.

TABLE 2. Effects of treatment in patients with cold and cholinergic urticaria

Treatment	No. of patients treated	No. of patients Cold urticaria Helped	Not helped	Cholinergic urticaria Helped	Not helped
Terfenadine	7	4	3	4	3
Hydroxyzine	3	3	0	3	0
Chlorpheniramine	6	3	3	3	3
Ketotifen	3	2	1	2	1
Cyproheptadine	5	1	4	2	3
Cimetidine + H_1 blocker	4	3	1	3	1
Desensitisation	4	2	1	0	1
Prednisolone	3	0	3	0	3
Danazol	2	NK	NK	2	0
Poldine (anticholinergic)	3	0	3	0	3

NK = Not known.

estimation in six patients are summarized in Figure 2. These were within the normal range, although two patients had values of inter-α-trypsin inhibitor well below one standard deviation from the mean.

The effects of treatments are summarized in Table 2. Results were often unsatisfactory and showed marked variability between patients. Desensitization to cold following the regimen of Bentley-Phillips[17] was attempted in three patients and achieved in two, only one of whom maintained this treatment. Desensitization to exercise by a strenuous regimen of exercise three times daily and hot baths twice daily for 2 weeks was unsuccessful in a single patient with cholinergic urticaria due to the short lived tolerance achieved.

Of the nine patients we were able to review, both types of urticaria had spontaneously resolved in three, 5, 6 and 26 years after onset, respectively.

DISCUSSION

Previous reports have suggested that the association of cold and cholinergic urticaria is rare,[8] but from our review of the literature and our own experience this association appears to occur frequently. As patients often react severely to cold, exercise, heat and emotion the combination is quite disabling, often prohibiting outdoor activities, heavy exercise and sport, particularly swimming.

The clinical distinction between cold and cholinergic urticaria was complicated by atypical features of the cold urticaria which occurred frequently in the present series. Four patients showed a hitherto unreported reaction to ice which resembled cholinergic urticaria with minute, discrete pinhead weals. We have not observed this pattern in patients who did not also have cholinergic urticaria. Unlike cholinergic urticaria, the reaction could not be prevented by prior application of hyoscine. Morphologically this reaction resembles 'local cold reflex urticaria'[18] which is thought to be mediated by a nervous reflex arc. However, unlike the lesions we describe, these 'cold' reflex lesions were seen around and not within the area of cold contact. In addition to heat induced cholinergic urticaria, two patients had generalized cold induced cholinergic urticaria[13] as recently reported by Farnham *et al.*[11]

Of the previously reported patients with cold and cholinergic urticaria one responded to doxepin and cimetidine[11] and another was successfully treated with high dose hydroxyzine together with cyproheptadine 16 mg daily.[8] In our series, cyproheptadine, a favoured drug in ordinary cold urticaria[12,19] proved effective in only one of five of the patients.[17] Terfenadine, hydroxyzine and ketotifen were useful in several patients and the addition of cimetidine was judged helpful in three out of four patients. Two patients with severe cholinergic urticaria responded well to danazol,[20] but this was given before they had cold urticaria. The effect of this drug in cold urticaria is not known.

The frequent association of the two forms of urticaria suggests common aetiological factors such as a circulating factor, possibly IgE, which recognizes epitopes revealed by both cold and heat stimuli. Cold-induced cholinergic morphology may be a consequence of neuronal involvement as in true cholinergic urticaria. In the cold induced cholinergic-like reactions, autonomic pathways which recognize and respond to cold stimuli (e.g. the pilomotor response) may be involved, triggered centrally in the generalized form and by an axon reflex in the localized form. In both cold and cholinergic urticaria α_1-antitrypsin deficiency has been found,[15] but as no such deficiency was found in our patients with both types, the combination does not appear to be related to this deficiency.

Data on the long-term outcome of the physical urticarias is lacking, but in the present series three of the nine patients who were traced and reassessed reported complete clearance of urticaria and this was confirmed in two by negative provocation tests. The urticaria cleared 5, 6 and 26 years after onset, respectively and remission was preceded by gradual improvement in frequency and severity of urticaria.

ACKNOWLEDGMENTS

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Darier's disease: an immunohistochemical study using monoclonal antibodies to human cytokeratins

S.M.BURGE*, D.A.FENTON†, R.P.R.DAWBER* AND I.M.LEIGH‡

*Department of Dermatology, The Slade Hospital, Headington, Oxford, †Dowling Skin Unit, St Thomas' Hospital, London and ‡Department of Dermatology, The London Hospital, Whitechapel, London, U.K.

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SUMMARY

The pathogenesis of Darier's disease was investigated by immunohistochemical staining of skin biopsies from involved and uninvolved skin in 14 patients, using monoclonal antibodies specific for keratins expressed in simple epithelia, stratified squamous epithelia and during skin specific differentiation as well as keratins expressed in mucosa and some benign epidermal hyperproliferative states.

Uninvolved perilesional skin from Darier's patients showed a normal profile of keratin expression, whereas in lesional skin there was apparent delay in the expression of the suprabasal skin specific keratins. Suprabasal keratins were not detected in basal cells, thus there was no true premature keratinization. The presence of hyperproliferative keratins was restricted to suprabasal cells in lesional skin. Four patients were receiving treatment with etretinate at the time of biopsy, but results in these patients did not differ from patients using topical treatments. Etretinate did not influence the profile of keratin expression in uninvolved or involved skin. The expression of type VII collagen was examined and was normal throughout uninvolved and lesional skin in Darier's disease.

All eucaryote cells have an intracellular cytoskeleton which is composed of actin-containing microfilaments, tubulin-containing microtubules and intermediate filaments, which vary in their properties. The 8 μm intermediate filaments of epithelial cells (keratin filaments or tonofilaments) are formed from different keratin sub-units. The keratins are a family of at least 19 water-insoluble polypeptides, which can be divided into two types or sub-families, encoded for by two distinct classes of genes.[1] Type I (sub-family A) are small acidic polypeptides with

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Correspondence: Dr S.M.Burge.

molecular weights between 40 and 56·5 kilodaltons (kd), while type II (sub-family B) are basic polypeptides with molecular weights between 52 and 67 kd.[2] A pair of keratins is sufficient for filament assembly, but at least one keratin from each sub-family is required.[3]

The composition of the filaments varies in different epithelial cell types and with the stage of differentiation or development, presumably changing the properties of the cytoskeleton to fulfil different needs e.g. cell division and migration.

Normal human epidermis contains four major keratins which are expressed sequentially in pairs during differentiation. An acidic 50 kd/basic 58 kd pair, present throughout the epidermis, are the only keratins expressed in basal cells, while an acidic 56·5 kd/basic 65–67 kd pair are expressed in differentiating cells, predominantly in the suprabasal layers.[4–6]

The expression of individual keratin polypeptides depends on the type of tissue and stage of differentiation or development. Although the site of subtle cellular changes in the composition of keratins cannot be detected using biochemical techniques, this can be explored with immunohistochemical methods.[7,8]

Darier's disease is an uncommon condition which has an autosomal dominant mode of inheritance. It is characterized by the presence of firm, greasy, crusted, brownish papules (Fig. 1); palmar pits and nail changes comprising V-shaped notches with linear bands. The histological features of the Darier lesion[9] include suprabasal epidermal cell separation (acantholysis) with the formation of fissures (lacunae) and characteristic 'prematurely keratinized'[10] dyskeratotic cells, and the disease has been classified as a disorder of keratinization.[10] The primary pathogenetic mechanism is unknown, but ultrastructural studies have demonstrated cytoplasmic vacuolization and breakdown of the complexes between keratin filaments (tonofilaments) and desmosomes with perinuclear aggregates of keratin filaments.[11–13] There is evidence from biochemical studies of the epidermal keratin extracted from the skin of patients that there are structural changes in the alpha-keratin polypeptides[14] and there may be abnormalities in the end-terminal non-alpha-helical regions of the polypeptides in diseased epidermis.

We have investigated keratin expression in 14 patients with Darier's disease employing murine monoclonal antibodies of known and restricted specificities. These detected keratins characteristic of simple epithelia (keratins 18 and 19[4]), stratified squamous epithelia (keratin 14), skin specific differentiation (keratin 10), as well as keratins expressed in mucosae and some epidermal hyperproliferative states e.g. psoriasis (keratin 16). In addition, the expression of type VII collagen, which is synthesized by keratinocytes, was examined using an antibody to the carboxy terminal region of the type VII collagen molecule.[15]

METHODS

Epidermal biopsies

Specimens were examined from nine males and five females with clinical characteristics of Darier's disease, which had been confirmed histologically. Five patients were taking etretinate 10–50 mg/day, and the remainder were using topical treatments (Table 1).

Punch biopsies of intact keratotic papules on the trunk or neck were snap frozen in liquid nitrogen and stored at −70°C until frozen sections were cut. Frozen normal skin samples obtained from surgical specimens were used as controls.

Monoclonal antibodies

The monoclonal antibodies used are listed in Table 2. Monoclonal antibody LH7:2 recognizes

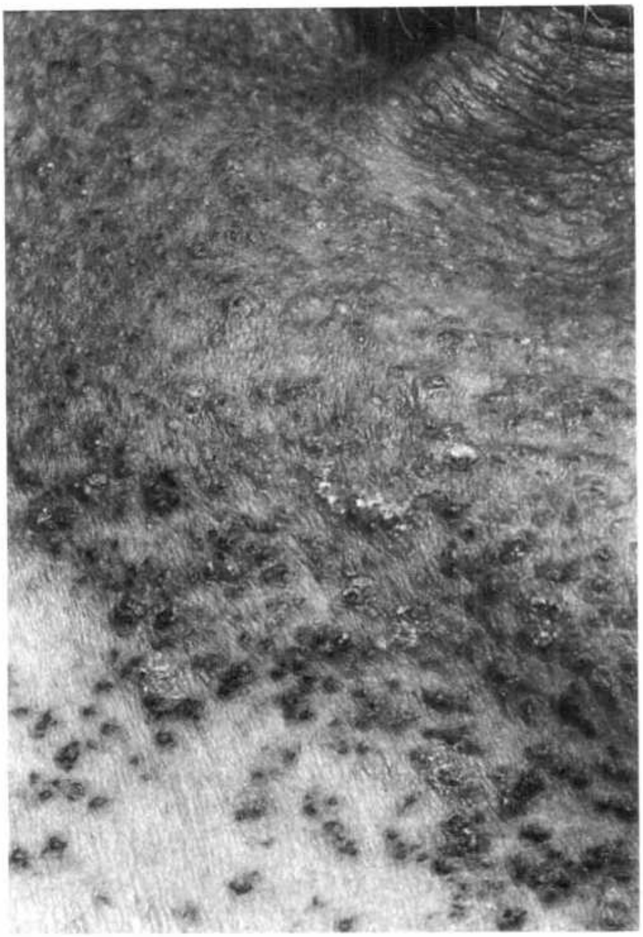

FIGURE 1. Brown, warty papules on the upper chest, which are a characteristic feature of Darier's disease.

the carboxy terminal region of the type VII collagen present in the lamina densa of stratified squamous epithelia.[15] The other antibodies have been characterized in previous studies.[6-8] The principal specificities of the monoclonal antibodies to keratins were as follows: LP34 recognizes a keratin common determinant expressed throughout human stratified squamous epithelia as well as simple epithelia. LE61 detects the 45 kd keratin (keratin 18[4]) present in simple epithelia, but absent *in vivo*, in normal keratinocytes, while LP2k reacts with a 40 kd keratin (keratin 19[4]) and is present in both simple epithelia and some sub-populations of keratinocytes, including those in a restricted area of outer root sheath.[6] LH6, LH8 and PAB 601 recognise determinants in the basal compartment (putative target: the 50 kd keratin, keratin 14[4]). LH P1, LHP2 and LH P3 detect the 56·5 kd suprabasal keratin (keratin 10[4]). LH12 is a recently developed monoclonal antibody which recognizes a determinant present diffusely in the basal epidermis, high suprabasal cells and upper dermis in psoriasis, but absent from normal epidermis. AE1 detects keratin 14 in basal cells of normal epidermis, but in addition detects the 48 kd keratin, keratin

TABLE 1. Details of patients studied

Patient no.	Sex	Age (years)	Biopsy site	Treatment
1	M	39	flank	Etretinate 25–50 mg/day Calmurid
2	M	40	chest	none
3	M	38	back	Etretinate 10 mg/day
4	M	27	flank	Ultralanum
5	M	60	flank	Ultralanum
6	M	70	neck	Vioform HC in flexures
7	M	67	trunk	Etretinate 25 mg/day
8	F	74	neck	Etretinate 50 mg/day
9	F	53	thigh	60% propylene glycol in aqueous cream
10	F	30	abdomen	none
11	M	31	neck	none
12	F	35	trunk	none
13	F	41	trunk	Roc Sun Bloc
14	M	33	trunk	Etretinate 50 mg/day stopped 3 weeks before the biopsy

16,[4] which is present suprabasally in mucosae, cultured keratinocytes and some hyperproliferative states.[5,8] LMM3 also detects this 'hyperproliferative' keratin.[16]

Immunohistology

Frozen, unfixed 8 μm sections of skin biopsies were examined. Monoclonal antibodies in undiluted culture supernatants were applied to the sections and incubated for 60 min at room temperature. Phosphate-buffered saline (PBS) was used in place of the monoclonal antibody to provide a negative control. Specimens were washed for 30 min with tap water and incubated with peroxidase-conjugated rabbit anti-mouse IgG (DAKOPatts) diluted 1:50 in Dulbeccos' modified Eagles medium (DMEM, Flow Lab Ltd) and 10% foetal calf serum for 60 min at room temperature. The sections were washed for a further 30 min and enzyme localization was visualized by incubation in 2·5 ml diaminobenzidine tetrahydrochloride (0·6 mg/ml) in PBS with 7·5 μl of hydrogen peroxide for 5–10 min, until the colour developed. Sections were washed again and counterstained with haematoxylin.

Immunofluorescence staining was carried out on some sections. The second antibody was fluorescein-labelled rabbit anti-mouse IgG (DAKOPatts) diluted 1:50 in PBS. Specimens were mounted in gelvatol 20–30 (Monsanto Petrochemicals) and examined by epi-fluorescence with a Zeiss Photomicroscope III. The sections were screened with all 14 monoclonal antibodies and then staining was repeated using selected antibodies to confirm the specificity. A number of specimens were incubated with a basal cell marker followed by a suprabasal marker in order to detect any sub-population of cells displaying no markers.

RESULTS

The results are summarized in Table 2. Using LH7:2, type VII collagen was detected uniformly in the basement membrane zone, outlining the finger-like papillary proliferations at the base of

TABLE 2. Monoclonal antibodies used in the study and results obtained with epidermis from patients with Darier's disease and normal control epidermis

Monoclonal antibody	Antigenic site	Reactivity in normal human epidermis	Reactivity in Darier's disease
LH7:2	Carboxy terminal region of type VII collagen molecule in lamina densa	Basement membrane zone	Basement membrane zone
LP34	Keratin common antigen	Panepidermal	Panepidermal
LE61	Keratin 18 (45 kd) present in simple epithelia	Merkel cells. No epidermal staining	No epidermal staining
LP2K	Simple epithelia and some basal subpopulations of stratified squamous epithelia (keratin 19; 40 kd)	Hair follicle—small set of cells in outer hair root sheath	No epidermal staining
LH6, LH8, PAB601	Basal cells (putative target keratin 14, 50 kd)	Single basal layer	Single basal layer around 'naevoid' downgrowths. Some supra-basal cells
LHP1, LHP2, LHP3	Keratin 10 (56·5 kd) present in differentiating cells	Uniformly in suprabasal cells above a single unstained basal layer	Suprabasal cells, but some unstained cells
LMM3	Hyperproliferative cells (keratin 16, 48 kd)	Hair follicle—outer hair root sheath	Faint suprabasal staining
AE1	Basal cells in normal skin (keratin 14, 50 kd) Suprabasal staining in hyperproliferative skin (keratin 16, 48 kd)	Single layer of basal cells	Suprabasal staining
LH12	2–3 basal cell layers, high suprabasal cells and upper dermis in psoriasis	None	Stain retained in parakeratotic plug and some suprabasal cells

the lesions (Fig. 2). The simple epithelial keratins (18 and 19) detected using LE61 and LP2K, were not expressed in normal or involved skin, while keratin common antigen (detected using LP34) was found throughout the epidermis in both uninvolved and involved skin. In uninvolved skin, a single layer of basal cells was stained with LH6, LH8 and PAB 601 (Fig. 3).

In involved skin, the papillary 'naevoid' downgrowths were also lined by a single layer of cells which stained with the basal cell markers LH6, LH8 and PAB 601 (Fig. 4). However, in addition, a number of acantholytic cells in a suprabasal position were stained with these markers (Fig. 4) and acantholytic cells surrounding suprabasal lacunae also expressed basal markers.

Antibodies which recognize keratin 10 in suprabasal cells (LHP1, LHP2, LHP3, LH13) left a

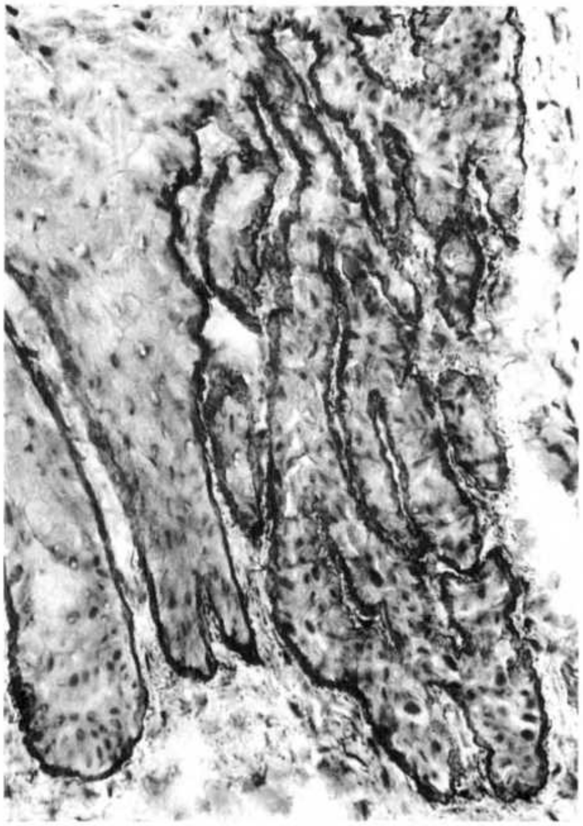

FIGURE 2. Immunohistochemical staining of involved skin in Darier's disease with LH7:2 which detects type VII collagen. Type VII collagen is expressed uniformly in the basement membrane zone outlining the papillary downgrowths. (Original × 400).

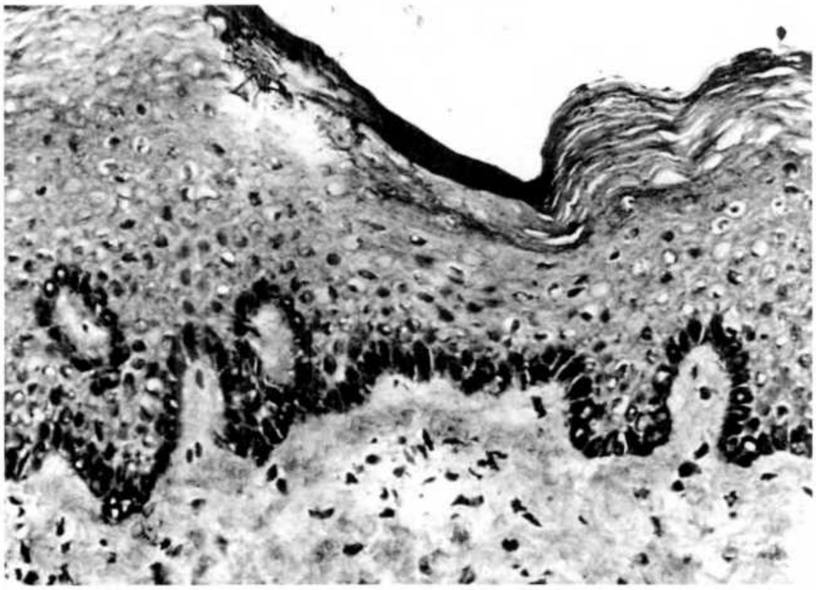

FIGURE 3. Immunohistochemical staining of normal skin with the basal cell marker LH8. A single layer of basal cells is stained. (Original × 400).

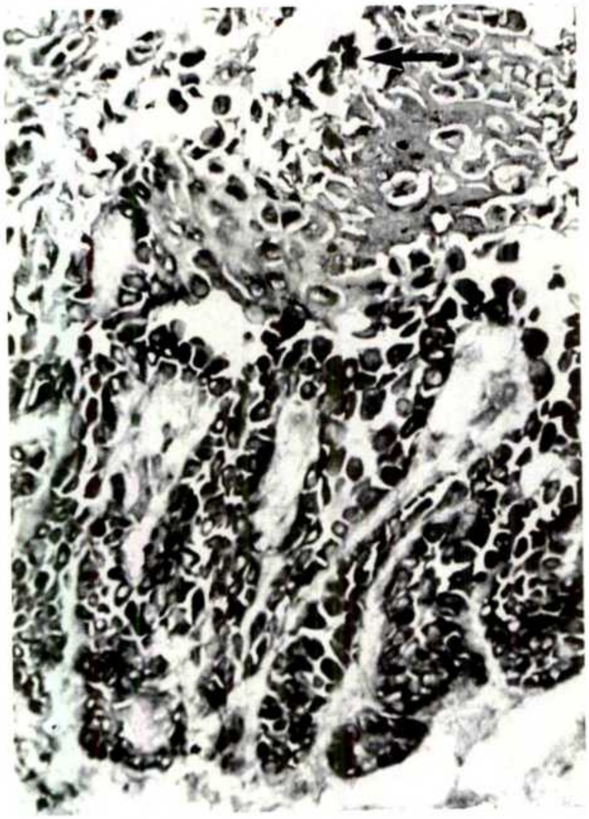

FIGURE 4. Immunohistochemical staining of a Darier's lesion with the basal cell marker LH8. Papillary downgrowths underlying the hyperkeratotic plug are lined with a single layer of cells expressing basal cell markers and in addition some suprabasal, acantholytic cells express this marker (arrow). (Original × 400).

single basal cell layer unstained in uninvolved perilesional skin (Fig. 5). However, in adjacent lesional skin although papillary projections were lined by unstained cells, there were many additional unstained acantholytic cells which were not in contact with the basal membrane (Figs 5 and 6). The majority of these cells were stained when the specimen was incubated with both a suprabasal marker and a basal marker. Many acantholytic cells did express keratin 10 normally (Fig. 6).

The antibody AE1 stained basal cells in uninvolved normal skin, but, in contrast, there was bright suprabasal staining in lesional skin (Fig. 7). LMM3 did not stain normal skin, but there was weak suprabasal staining, similar to that seen with AE1, in involved skin. Antibody LH12 was bound to a determinant present in the cytoplasm of cells in the parakeratotic plug and some suprabasal cells (Fig. 8). There was no staining of adjacent normal stratum corneum or epidermis.

The expression of markers in the four patients taking etretinate was similar to that in the seven patients using topical, non-retinoid, treatments.

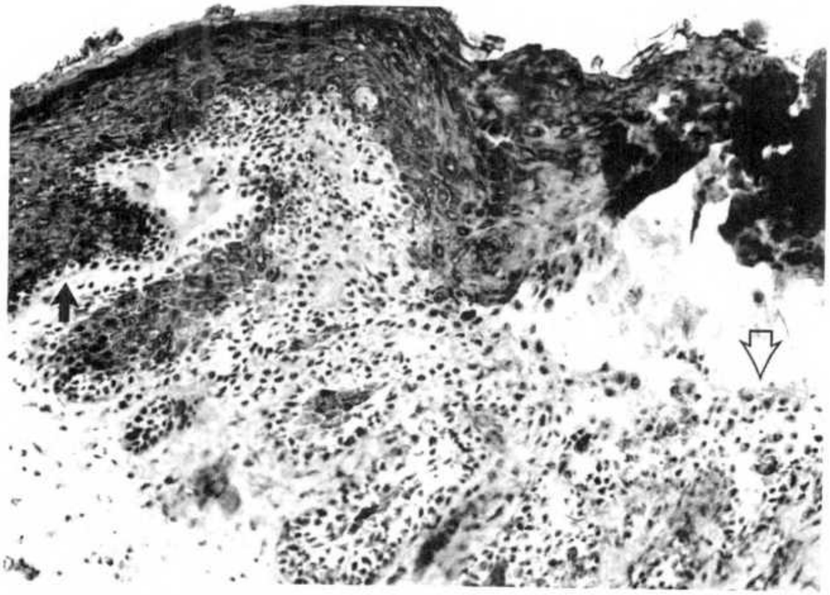

FIGURE 5. Immunohistochemical staining of a Darier's lesion with the suprabasal cell marker LHP2. A single basal layer is unstained in normal skin (solid arrow). In lesional skin the papillary projections are lined by unstained basal cells but in addition there are many unstained suprabasal acantholytic cells (open arrow). (Original × 400).

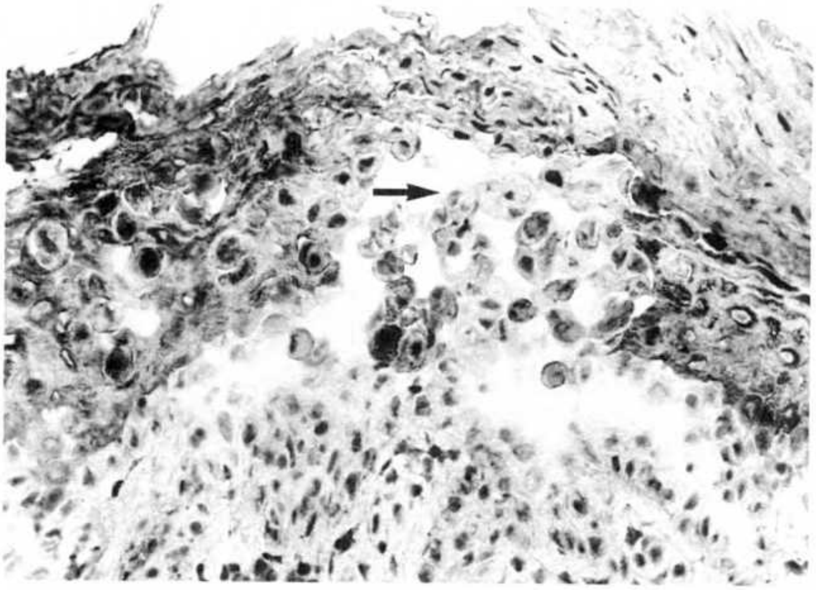

FIGURE 6. Immunohistochemical staining of a Darier's lesion with the suprabasal marker, LHP2. Although many of the suprabasal acantholytic cells express this marker, there are groups of unstained cells in a high suprabasal position (arrow). The basal layer is unstained. (Original × 400).

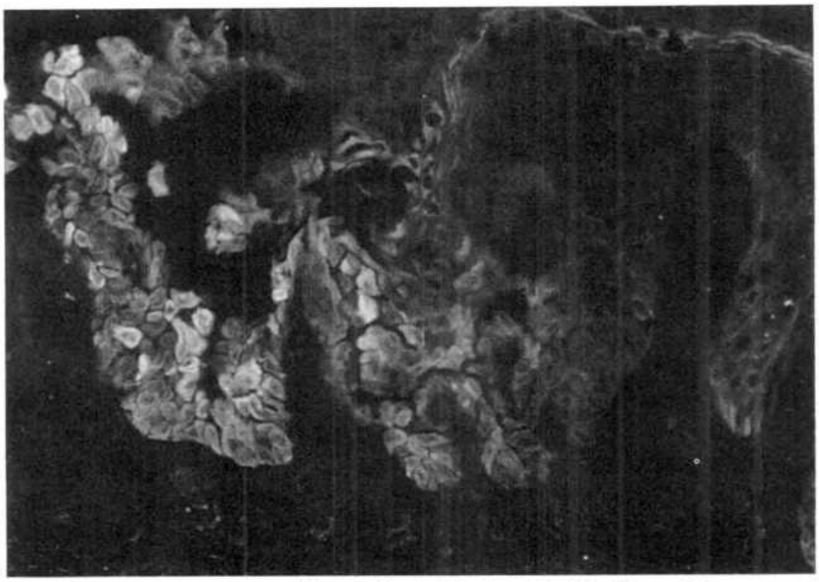

FIGURE 7. Immunofluorescence staining of a Darier's lesion with AE1. There is intense suprabasal staining. Adjacent normal epidermis displays faint basal staining with non-specific fluorescence of the stratum corneum. (Original × 400).

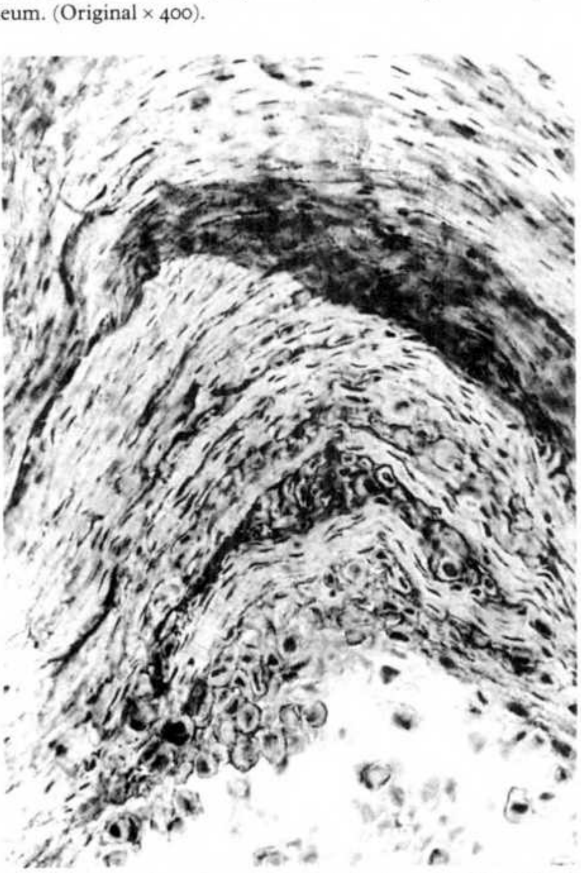

FIGURE 8. Immunohistochemical staining of a Darier's lesion with LH2. There is expression of a determinant in both the parakeratotic plug and some suprabasal cells. There is no staining of adjacent normal stratum corneum or epidermis. (Original × 400).

DISCUSSION

There was uniform expression of type VII collagen in the basement membrane zone throughout normal and involved skin, indicating that keratinocytes are capable of synthesizing and secreting type VII collagen in Darier's disease.

Uninvolved skin, adjacent to lesions, showed a normal profile of keratin expression, in that the expression of the basal cell markers detected by LH6, LH8 and PAB 601, was restricted to a single basal cell layer, while keratin 10 detected by LHP1 LHP2, LHP3 and LHP13, was expressed by suprabasal cells above a single layer of unstained basal cells. We could not detect any subclinical pathology which could predispose to acantholysis.

There was normal expression of keratin common antigen throughout the specimens. Keratins 18 and 19 are present in many simple, non-stratifying epithelia (intestine, gall bladder and various glands),[4] and keratin 19, detected by LP2K, is also expressed in a restricted zone of the outer hair root sheath.[6] We were unable to detect these keratins in uninvolved or lesional skin.

The convoluted 'budding' at the base of many of the lesions was composed of cells which were morphologically basal, with a columnar shape and dark-staining nuclei. The basal nature of the cells was confirmed by the immunohistochemical results, which demonstrated that the cells expressed basal cell markers, but did not express keratin 10. However, the expression of basal markers was not restricted to cells in the basal layer and, unlike adjacent normal skin, this marker was also expressed by some acantholytic suprabasal cells. These cells were detached from the basement membrane and this unique finding was not secondary to cross-cutting giving the cells a false suprabasal location (Figs 6 and 7). Suprabasal lacunae were lined by cells expressing these basal markers.

The expression of basal markers by suprabasal cells could be secondary to a delay in the synthesis or suprabasal expression of keratin 10 and may be associated with an abnormality in the keratin filament-desmosome complexes, which could predispose to acantholysis. However, if this is the primary abnormality in Darier's disease, one would expect the majority of the acantholytic and dyskeratotic cells to continue to express the basal keratin markers, because there is evidence from studies of the incorporation of uridine, leucine and histidine into keratinocytes in Darier's disease, that RNA and protein synthesis ceases once acantholysis commences.[17] In fact, the majority of the suprabasal cells, including acantholytic and dyskeratotic cells, expressed keratin 10 in a normal fashion. Presumably the acantholytic process involved these cells after expression of this keratin had commenced. Rather than a true abnormality in differentiation, i.e. the synthesis of skin specific differentiation keratin filaments, there may be an alteration in target epitopes. Abnormal aggregations of keratin filaments or degeneration in acantholytic cells may either facilitate the binding of basal cell markers by unmasking epitopes on keratin 14, which is present throughout the epidermis,[5] or impede the binding of suprabasal markers to keratin 10.

Immunohistochemical studies in a rat mammary epithelial cell line have demonstrated that cell to cell contact and desmosome formation are a pre-requisite for the formation of keratin filaments.[18] If desmosomes are disrupted there is a rapid breakdown in filaments with a change in antigenicity. Although there is no evidence from *in vitro* studies of cultured human keratinocytes that an absence of desmosomes prevents the synthesis of keratin filaments, *in vivo* a breakdown of desmosomes might influence the structural conformation of keratins.

The basal cell layer in normal skin is composed of a heterogeneous population of cells only some of which are proliferating. Others may be post-mitotic and maturing or resting.[19] A proportion (8%) of basal cells which are rapidly proliferating, may express suprabasal markers of differentiation despite their basal position,[20] but we were unable to demonstrate a subpopulation of differentiating basal cells with the monoclonal antibodies employed in the present study. The term 'premature' keratinization[10] which implies premature synthesis of skin specific differentiation keratin filaments, is inappropriate to describe the dyskeratosis in Darier's disease.

The involved skin in patients with Darier's disease has an increased labelling index[21] which may reflect hyperproliferation, but unfortunately, there are no cell birth rate estimates to provide a reliable guide to proliferative activity.[22] The suprabasal staining which we obtained in lesions using monoclonal antibodies AE1 and LMM3 may also reflect hyperproliferation or 'wound healing'. Normally antibody AE1 detects keratin 14 in the basal layer of the epidermis.[5] However, there is a striking difference in the staining pattern of AE1 in normal palate, normal heel and some benign hyperproliferative conditions e.g. psoriasis, gingiva with chronic inflammation, epidermis after tape stripping, seborrhoeic keratoses and actinic keratoses. In these situations, AE1 shows a suprabasal staining pattern. The change in AE1 immunoreactivity is associated with the presence of keratin 16.[7,23,24] LMM3, unlike AE1 does not show any reactivity in normal skin, but detects keratin 16 in psoriasis, keratinocytes at the edge of healing wounds and in other benign hyperproliferative states.[15]

The suprabasal staining in Darier's disease probably reflects the presence of this keratin and may indicate an alteration in differentiation in association with hyperproliferation. Keratin 16 is normally paired with a 56 kd polypeptide (keratin 6). This pair of keratins is also detected in keratinocytes in culture. The mRNAs encoding keratin 6 are present in normal skin and are rapidly induced in hyperproliferative states.[25] The altered expression of keratins may aid cell mobility and plasticity in situations where rapid cell division or migration are important.

The monoclonal antibody LH12 reacts with a 46 kd protein present diffusely in the basal cell layers in psoriasis and in the stratum corneum (Leigh, unpublished). The striking reactivity of the abnormal cornified plug in Darier's disease may be parallel to the reactivity in psoriasis and reflect hyperproliferation or trapping of a soluble protein.

Although there may be hyperproliferation in Darier's disease, it is unlikely that this is responsible for the disturbance in the expression of basal and suprabasal markers. In psoriatic hyperproliferation the basal-suprabasal division is intact with a single layer of basal cells expressing basal markers.[26]

The sequence of events leading to acantholysis is unclear, but the breakdown of the keratin filament-desmosome complex with dyskeratosis may not be the primary abnormality. There is evidence from cell culture work that keratinocytes from patients with Darier's disease secrete a 'cell dissociating factor,' possibly a protease, which induces cell separation.[27,28]

It has also been suggested that increased proteolysis could be responsible for biochemical abnormalities in the keratin profile.[14] Similarly, focal proteolytic activity could alter the epitopes detected by monoclonal antibodies in Darier's disease.

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Site-specific melanocytic naevus counts as predictors of whole body naevi

J.S.C.ENGLISH*, A.J.SWERDLOW†, R.M.MACKIE,* C.J.O'DOHERTY‡, J.A.A.HUNTER‡, J.CLARK†, AND D.J.HOLE§

* Departments of Dermatology, and †Community Medicine, University of Glasgow, ‡Department of Dermatology, University of Edinburgh and §West of Scotland Cancer Surveillance Unit, Glasgow, U.K.

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SUMMARY

Counts of melanocytic naevi of 2 mm or greater diameter on all body sites were conducted in 197 Caucasian adults in Scotland. Mean naevus numbers were greater in young than in older adults, and in females than in males. Naevus counts on the upper limbs, lower limbs, and trunk were strongly and significantly correlated with each other and with whole body counts. Thus, where whole body counts are impractical, counting naevi at any of these individual sites should provide a satisfactory method for studying naevus distribution in large populations.

The rapid rise in the incidence and mortality of malignant melanoma over recent years has led to increased interest in the epidemiology of malignant melanoma and especially its relationship to melanocytic naevi. Several recent case-control studies have shown that naevus counts are a strong risk factor for melanoma,[1–4] but relatively little is known about the distribution of and the risk factors for naevi. There have been few studies of whole body naevus counts in populations; such counts are time consuming and can be difficult to conduct in non-hospitalized patients. In the present study we have examined the relationship between naevus numbers at different sites, and also between site-specific naevus counts and whole-body counts, in a Caucasian population in Scotland.

METHODS

The subjects of the present analysis were the control group (i.e. patients without melanoma) from a case-control study investigating various possible risk factors for melanoma, details of which have been published previously.[4] The subjects consisted of 197 Caucasian patients (120 females and 77 males), with a wide range of admission diagnoses, the most common being hypertension (55 patients), psoriasis (31 patients) and fractures or dislocations (20 patients).

Correspondence: Dr J.S.C.English, St. John's Hospital for Diseases of the Skin, 5 Lisle Street, London WC2H 7BJ, U.K.

Sixty eight per cent of patients were interviewed and examined in Glasgow and 32% in Edinburgh.

Benign melanocytic naevi greater than 2 mm diameter were counted by trained dermatologists on all body sites except the scalp and genitalia. Skin lesions were judged to be naevi for the purpose of this study if they were brown and not obviously freckles, lentigines, or basal cell papillomas. For recording of naevi the body was divided into the following anatomical regions: upper limbs, lower limbs, trunk excluding genitalia, neck, face, palms, and soles. Partial correlation coefficients adjusted for age were calculated between naevus counts at each of the sites, and between site-specific counts and overall counts.

RESULTS

Mean counts of naevi on the whole body, and on each site where there were substantial numbers, decreased with age in adults (Table 1). At each age, naevus counts were markedly greater in women than in men for the whole body and for the upper limbs and lower limbs; for trunk naevi, however, there was a male excess at younger ages (Table 1).

Correlating naevus numbers on different body sites with each other, strong, highly significant correlations were seen in each sex between naevi of the trunk and upper limbs, of the trunk and lower limbs, and of the upper limbs and lower limbs (Table 2). Neck and face naevi generally showed moderate correlations with naevi at other sites (Table 3), whilst palm and sole naevi showed lesser correlations—in all instances 0·25 or less (data not shown).

In each sex there were strong, highly significant positive correlations between whole-body naevus counts and counts on each of the upper limbs, lower limbs, and trunk ($P < 0{\cdot}001$) (Table 3). Weaker, although in several instances still highly significant, correlations with whole-body counts were seen for naevus numbers on the neck, face, palms, and soles.

TABLE 1. Mean naevus counts in 197 Caucasian subjects, broken down by sex, age group and body site

	Females		Males	
	Age < 50 years (n = 59)	Age ≥ 50 years (n = 60)	Age < 50 years (n = 41)	Age ≥ 50 years (n = 37)
Site	Mean count (%)	Mean count (%)	Mean count (%)	Mean count (%)
Palms	0·12 (0·6)	0·02 (0·3)	0·04 (0·3)	0 (0)
Soles	0·03 (0·2)	0·07 (0·9)	0·05 (0·4)	0 (0)
Upper limbs	9·6 (46·7)	2·8 (36·7)	5·3 (37·4)	1·4 (42·0)
Lower limbs	6·9 (33·6)	2·2 (28·8)	3·1 (21·8)	0·62 (18·2)
Trunk	2·6 (12·7)	1·6 (21·1)	4·2 (29·6)	1·0 (30·4)
Face and neck	1·3 (6·2)	0·9 (12·2)	1·5 (10·5)	0·32 (9·4)
Total	20·5 (100)	7·6 (100)	14·2 (100)	3·4 (100)

TABLE 2. Partial correlation coefficients, adjusted for age, of naevus counts on selected anatomical sites with counts on other sites

	Partial correlation coefficient	
Sites compared	Males	Females
Trunk and upper limbs	0·71	0·70
lower limbs	0·61	0·61
neck	0·23	0·54
face	0·22	0·48
Upper limbs and lower limbs	0·55	0·71
neck	0·57	0·57
face	0·22	0·35
Lower limbs and neck	0·23	0·45
face	0·46	0·28
Neck and face	0·23	0·55

TABLE 3. Partial correlation coefficients, adjusted for age, of naevus counts on different anatomical sites with whole-body naevus counts

	Partial correlation coefficient	
Site	Females	Males
Trunk	0·82*	0·90*
Upper limbs	0·91*	0·85*
Lower limbs	0·91*	0·83*
Neck	0·62*	0·41*
Face	0·45*	0·41*
Soles	0·29*	0·11
Palms	0·09	0·11

* Statistically significant; $P < 0{\cdot}001$.

DISCUSSION

The demographic and site distribution of naevus numbers in the present subjects were generally similar to those in several previous studies of Caucasians,[5–7] where mean numbers of naevi in adults were found to decrease with age, and were greater for females than males on the upper limbs and head and neck, but greater for males than females on the trunk. There have been relatively few studies presenting data on whole-body naevus counts,[5–7] however, and little is known of many aspects of the descriptive epidemiology of naevi—for instance regarding geographical variation and time trends. Study of naevi is hampered by the practical problems of whole-body counts—they are time consuming to undertake, and it can be difficult to gain agreement for them and to conduct them in the general population. For these reasons some studies have restricted somewhat the sites examined—Cooke *et al.*[6] for example, excluded the abdomen, buttocks and anterior chest from their counts. The effect of counting only on restricted areas of the body has not been examined however, and there appear to have been no previous analyses published of the correlations between site-specific counts or of the correlations between these and whole-body counts.

Our study showed that naevus numbers on each of the upper limbs, lower limbs and trunk, correlated strongly with each other, indicating that they may share a similar aetiology. Numbers of naevi at these sites also correlated strongly with whole-body numbers, suggesting that counts on any one of the sites—most simply the upper limbs—should give a reasonable assessment of overall naevus numbers in population studies when whole-body counts are impractical. This gives a practical method to extend the very limited current knowledge of naevus distribution and risk factors. It should be noted, however, that the results were for counts of naevi 2 mm or greater in diameter in a Caucasian population. Separate investigation is needed of the relationship of site-specific naevus numbers to each other and to whole-body numbers, for all naevi and for large naevi, and for counts in non-Caucasians.

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Localization of cutaneous antigens by the immunogold-silver staining technique

M.H.ALLEN, M.J.TIDMAN AND D.M.MACDONALD

Laboratory of Applied Dermatopathology, United Medical & Dental Schools of Guy's and St. Thomas's Hospitals (Guy's Campus), London, U.K.

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SUMMARY

Immunogold-silver staining (IGSS) was evaluated for the localization of cutaneous antigens in frozen and paraffin-embedded tissue, employing antibodies to a variety of intracellular, cell-surface and extracellular epitopes in an indirect immunogold reaction, followed by silver enhancement. The principal advantages of IGSS are the avoidance of toxic reagents and the production of a silver precipitate that is permanent, clearly visible and of sufficient contrast with the reaction products of alternative immunolocalizing techniques to be of value in double-labelling procedures. It seems to localize antigenic determinants well, but does not appear to be particularly sensitive, especially for the demonstration of antibodies to extracellular constituents. Limitations of IGSS include the propensity to non-specific staining, and the need to vary the duration of the development stage during silver enhancement.

Following its introduction in 1971,[1] colloidal gold has become firmly established as an immunoelectron microscopic label. Its advantages include sensitivity, the excellent localization of antigenic determinants, the capacity for multi-labelling procedures by the use of gold particles of different sizes,[2] and the potential for quantification.[3] However, as a light microscopic label, colloidal gold suffers from the major drawback of having a subtle red or pink colour when viewed by transmitted light.

Immunogold-silver staining (IGSS) is a relatively new technique[4] by which the gold label in immunocytochemical reactions is enhanced by the controlled precipitation of metallic silver onto the gold particles, thereby increasing their size and imparting a dense brown or black colour which greatly improves the light microscopic appearance. It involves the reduction of silver ions in the presence of a reducing agent, usually hydroquinone. This reaction is catalysed initially by the gold and subsequently by the metallic silver which accumulates as a shell around the gold particles.

Correspondence: Dr D.M.MacDonald, Department of Dermatology, Guy's Hospital, St. Thomas' Street, London SE1 9RT, U.K.

IGSS has been demonstrated to be useful in a number of situations,[5–7] but it has not yet been fully assessed for use with cutaneous tissue, although preliminary reports have suggested its potential.[8–10] The aim of the present study was to evaluate the IGSS technique for the light microscopic localization of cutaneous antigens in frozen and paraffin-embedded skin sections.

METHODS

A panel of primary antibodies to a variety of intracellular, cell-surface and extracellular constituents (Table 1), was employed in a standard indirect immunocytochemical reaction on sections of frozen or paraffin-embedded skin, using a secondary antibody conjugated with colloidal gold. The primary antibodies were selected on the basis of being in routine use in our laboratory throughout the 18-month period of the study.

Cryostat sections (5 μm) were air-dried, fixed in acetone for 5 min, and then immersed in undiluted normal swine serum, before incubation with the primary antibody, at the optimum dilution as determined for individual antibodies (Table 1), for 60 min at room temperature. The sections were washed with Tris buffered saline (TBS) at pH 7·6, followed by exposure to the gold-conjugated secondary antibody (goat immunoglobulin against either mouse immunoglobulin at a dilution of 1 : 10 or rabbit immunoglobulin at a dilution of 1 : 20) for 30 min, washed thoroughly with two changes of TBS and then fixed in 2% glutaraldehyde for 15 min.

After further thorough washing with two changes of TBS and three changes of distilled water, silver enhancement was accomplished using a commercial kit, 'IntenSE' (Janssen Pharmaceuticals Ltd., Beerse, Belgium), which incorporates silver lactate as the ion source and hydroquinone as the reducing agent. This combination allows the silver precipitate to be readily controlled by altering the duration of development. The intensification stage was conducted within a closed laboratory cupboard for protection from light. The silver was then fixed and, after further washing, the tissue lightly counterstained with haemalum and mounted.

TABLE 1. Details of the primary antibodies used in the study

Antibody	Dilution on frozen skin	Source	Localization of antigen
Cell surface OKT 6	1:20	Ortho[a]	Langerhans cells
Leu 1	1:20	Becton-Dickinson[b]	T lymphocytes
Leu 2a	1:25	Becton-Dickinson	T lymphocytes (suppressor)
Intracellular LP 34	1:30	Personal gift[c]	Common keratin marker
LHP3	1:30	Personal gift	Keratin 10
anti-alpha-1-antichymotrypsin	1:50	Dako[d]	Macrophages
anti-alpha-1-antitrypsin	1:200	Dako	Macrophages
anti-lysozyme	1:50	Dako	Macrophages
Extracellular anti-type IV collagen	1:30	Personal gift	Basement membrane
LH7:2	1:20	Personal gift	Basement membrane (type VII collagen)
GDA-J/F3	1:10	Personal gift[e]	Basement membrane

[a] Ortho-Diagnostic Systems Ltd., U.K.

[b] Becton-Dickinson Monoclonal Center Inc., U.S.A.

[c] Personal gift, from Dr I.M.Leigh, The London Hospital, U.K.

[d] Dakopatts, Denmark.

[e] Personal gift, from Dr A.Jassim, The London Hospital, U.K.

The method for paraffin-embedded sections was virtually identical, except that, after dewaxing and rehydration, the sections were treated with pronase (Sigma Chemical Co. Ltd., Poole, U.K.) 0·5 mg/ml, for 10 min at 37°C.

For each primary antibody, localization with IGSS was compared with a standard indirect immunoperoxidase technique.[11] A comparison was also made between results obtained by the use of secondary antibody conjugated with gold particles of two different sizes—5 nm and 15 nm diameter.

Controls consisted of the omission of the primary antibody, the use of irrelevant antibodies and, where appropriate, preabsorption of the antibody with antigen.

RESULTS

Demonstration of antibodies to cell-surface antigens (Fig. 1) and to intracellular antigens (Fig. 2) using IGSS compared favourably with the immunoperoxidase technique. The silver deposit was prominent and contrasted strongly with the background. Immunostaining of keratin epitopes by IGSS on frozen skin consistently demonstrated a focal accumulation of reaction product within individual cells (Fig. 2) rather than the diffuse deposition present with immunoperoxidase and with IGSS on wax-embedded material. The localization of extracellular antigens with IGSS was disappointing. None of the basement membrane constituents was satisfactorily demonstrated by IGSS, and the immunoperoxidase technique was clearly superior with these particular antibodies. Progressive dilution of a selection of the primary antibodies to determine the dilution at which immunostaining on frozen tissue disappeared confirmed the relative insensitivity of IGSS with the basement membrane antibodies (Table 2). Use of a secondary antibody conjugated with the 5 nm diameter gold particle resulted in considerably better immunolocalization than the 15 nm gold label.

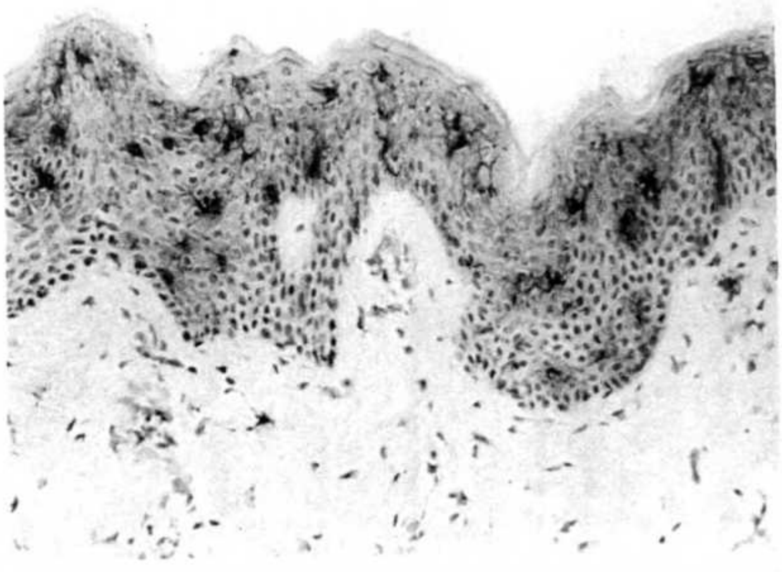

FIGURE 1. OKT6 positive dendritic cells in the epidermis of a frozen skin section stained with the immunogold-silver technique (original × 50).

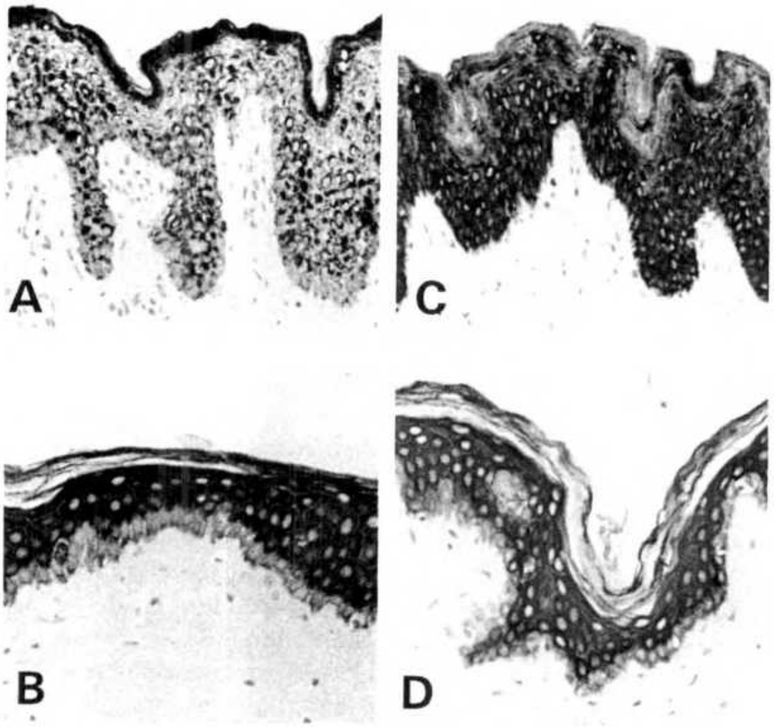

FIGURE 2. Localization of LP34, a marker of a common keratin epitope, by immunogold-silver staining on (a) frozen skin and (b) paraffin-embedded skin; compared with peroxidase labelling on (c) frozen skin and (d) paraffin-embedded skin. (Original × 50).

TABLE 2. Primary antibody dilutions at which immunostaining disappeared with IGSS and immunoperoxidase techniques on frozen skin sections

	Dilution	
Antibody	IGSS	Immunoperoxidase
OKT6	1:5120	1:5120
LP34	1:7620	1:3840
LH7:2	1:20	1:1280
anti-type IV collagen	1:480	1:7280

DISCUSSION

The IGSS technique has a number of advantageous features. It is relatively simple to perform, avoids the use of toxic reagents and is inexpensive. The silver precipitate is permanent, readily visible and affords sufficient contrast to allow concomitant use of routine histological stains. Furthermore, there is no problem, as there may be with peroxidase or alkaline phosphatase labelling, of confusion caused by endogenous tissue constituents. The superior results obtained with the 5 nm gold-label are in accordance with previous observations[12,13] and are probably related to the greater ability of the smaller particles to penetrate the tissue and thereby produce a more uniform precipitation of the silver reaction product.

The focal accumulation of reaction product within individual cells with keratin antibodies on frozen tissue may be interpreted as superior antigenic localization by the IGSS method. However, comparison of IGSS and immunoperoxidase techniques by means of the dilutions of the primary antibodies at which immunostaining disappeared suggests that, contrary to reports in other situations,[4,12,13] IGSS is not particularly sensitive when used on skin and is considerably less sensitive than immunoperoxidase in the demonstration of extracellular constituents.

The pronase step in paraffin embedded sections, although not essential for localizing the antigens under investigation in the present study, significantly enhances the sensitivity of the technique. Many studies of IGSS on paraffin sections have stressed the improvement in sensitivity consequent on including Lugol's iodine treatment prior to the application of the antibodies,[4,5,12] an effect possibly related to its mild oxidizing properties.[4] However, in our experience any marginal improvement with Lugol's iodine is at the expense of a marked deterioration in non-specific background staining.

The presence of non-specific background staining may be a problem with IGSS, but can be minimized by careful attention to the washing stages in order to remove non-specifically bound gold and halides in the tissue that might otherwise interfere with the enhancement stage. In addition, scrupulous cleanliness of glassware and purity of water is important and, ideally, deionized and double distilled water should be used. Another limitation of the technique is the variable time taken for the desired level of silver development, the optimum having to be determined on a trial-and-error basis.

We conclude that, although IGSS suffers from certain drawbacks, it may prove to have a significant role in cutaneous immunocytochemistry, particularly in double-labelling procedures, because of the stark contrast between the silver deposit and the reaction products of other immunolocalizing techniques.

ACKNOWLEDGMENTS

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A histological and immunocytochemical study of early acne lesions

J.F.B.NORRIS AND W.J.CUNLIFFE

Department of Dermatology, The General Infirmary at Leeds, Leeds LS1 3EX, U.K.

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SUMMARY

We examined 69 biopsies of acne lesions known to be 6, 24 or 72 h old and found that the lymphocyte was the predominant cell at 6 and 24 h. The helper: suppressor T cell ratio in the inflammatory infiltrate was 2·8 : 1. Polymorphonuclear leukocytes were increasingly seen at 24 and 72 h and were associated with disruption of the duct. We hypothesize that this early lymphocytic infiltrate represents a cell mediated immune response to an antigen within the duct lumen.

Current theory on the development of inflamed acne lesions contends that all lesions begin with non-inflamed microcomedones which eventually rupture and release inflammatory stimuli into the adjacent dermis.[1] The cellular mediator of this inflammation has been regarded as the polymorphonuclear leukocyte (PMN); Kligman[1] describes them accumulating focally outside the duct and then wandering through the epithelial wall to pool on the inner surface. The involved ductal epithelium becomes necrotic and PMNs become massed inside the duct as well as around it.

Research in the acne field has attempted to take account of this emphasis on the PMN; the effect of antibiotics on acne has been explained in terms of inhibition of leukocyte chemotactic factor;[2] and early PMN infiltrates are regarded as a criterion for animal models of acne.[3]

Considerable evidence has now accumulated to suggest that the host immunological response plays an important role in acne. Patients with acne have been shown to have elevated antibody titres to both *Propionibacterium acnes*,[4] and also to *P. acnes* cell wall carbohydrate,[5] increased lymphocyte transformation to *P. acnes* antigen,[6] delayed skin test reactivity to *P. acnes*,[7] significantly elevated IgG levels,[8] an activated helper T cell population,[9] and complement deposition in the walls of dermal blood vessels.[10,11] These findings suggest that both humoral and cell-mediated hypersensitivity reactions are involved in the inflammatory process of acne. If this is so, then the lymphocyte might be expected to play a more dominant role than the PMN in the early acne lesion.

Correspondence: Dr J.F.B.Norris.

The purpose of the present work was to investigate the inflammatory infiltrate in early acne lesions over a 72 h time course.

METHODS

Sixty-nine subjects (42 males and 27 females) aged between 17 and 24 years, with moderately severe acne on the Leeds grading scale were biopsied on the upper or mid back. They had been off all therapy for 1 month.

One problem with biopsying early acne lesions is deciding what constitutes an early lesion, because 'small' is not necessarily 'early'. To overcome this difficulty we used a clear acrylic sheet which was placed on the patient's back and all the underlying inflammatory lesions were marked. Twenty-four hours later this same sheet was replaced and any new lesions were easily seen. A 24-h lesion thus identified could be biopsied immediately or left to mature into a 72-h lesion. In a similar fashion 6-h lesions were also obtained. The biopsies were 4 mm punches which were routinely fixed, serially sectioned at 4 μm intervals, and stained with haematoxylin and eosin.

The cellular infiltrate was classified both in terms of cellular type (lymphoid, PMN or mixed), and intensity (minimal, moderate, marked or intense). Three areas were assessed—perivascular infiltrates, focal infiltrates adjacent to the duct wall, and background infiltrates in the general region of the whole gland. The term lymphoid is used here to denote small round cells which are difficult to classify as lymphocytic or monocytic by light microscopy.

The duct wall was examined for spongiosis or rupture, and the presence and type of comedone was assessed.

A further 14 24-h biopsies were snap frozen in liquid nitrogen and studied with a range of monoclonal antibodies: T28 as a pan T cell marker, OKT4 to detect helper/inducer T cells, OKT8 to detect suppressor/cytotoxic T cells, and OKT6 to detect Langerhans cells.

RESULTS

Sixty nine biopsies were obtained as follows: 15 at 6 h, 36 at 24 h and 18 at 72 h. The lesions biopsied had the morphology of small papules with a minimal surrounding erythematous flare.

The numbers of biopsies with perivascular infiltrates are listed in Table 1. All except two of the biopsies had a perivascular infiltrate which was predominantly lymphoid and most marked at 6 h; a mixed perivascular infiltrate, indicating the presence of PMNs, was only seen at 24 and 72 h. It should be emphasized that in these and all following results the lymphoid element always outnumbered the PMN element in the mixed infiltrates.

TABLE 1. Numbers of acne lesions biopsied at 6 h, 24 h and 72 h showing a perivascular infiltrate

	No. of lesions		
	6 h	24 h	72 h
Lesions with infiltrate	14 of 15	35 of 36	18 of 18
Lymphoid infiltrate	14 (100%)	27 (77%)	12 (67%)
Mixed infiltrate	0	8 (23%)	6 (33%)

The numbers of biopsies with a periductal focal infiltrate are listed in Table 2. The biopsies showed a low incidence of periductal focal infiltration which was mainly lymphoid in nature, but there were increasing numbers of PMNs in the 24 and 72 h biopsies.

The composition of the background infiltrate is listed in Table 3. A background infiltrate occurred in almost half of the biopsies; it was mainly lymphoid in nature in the 6 and 24 h biopsies, but at 72 h the PMN element had become prominent.

The majority of lymphoid infiltrates were classified as moderate with no obvious differences at any of the three time points. The PMN infiltrates, however, were generally moderate at 24 h increasing to marked or intense at 72 h.

The numbers of biopsies showing duct spongiosis or rupture or both are listed in Table 4. The commonest finding was spongiosis alone, or spongiosis in association with rupture; rupture without associated spongiosis was uncommon. A minority of the biopsies (39%) had no evidence of duct disruption.

TABLE 2. Numbers of acne lesions showing a periductal focal infiltrate

	6 h	24 h	72 h
No. of lesions with infiltrate	2 of 15 (13%)	11 of 36 (31%)	3 of 18 (16%)
Infiltrate lymphoid	1	6	1
mixed	1	3	0
PMN	0	2	2

TABLE 3. Numbers of acne lesions showing a background infiltrate

	6 h	24 h	72 h
No. of lesions with infiltrate	7 of 15 (47%)	19 of 36 (53%)	9 of 18 (50%)
Infiltrate lymphoid	5 (71%)	13 (68%)	4 (44%)
mixed	2 (29%)	6 (32%)	5 (56%)

TABLE 4. Numbers of acne lesions showing duct rupture and spongiosis

	6 h	24 h	72 h	
Spongiosis alone	3	11	5	
Rupture alone	2	2	3	
Both	0	12	4	
Total	5 of 15 (33%)	25 of 36 (69%)	12 of 18 (67%)	Overall total 42 of 69 (61%)

TABLE 5. Presence and type of comedones in acne lesions studied

Type of comedone	Micro	Open	Closed	None
Number	36 (52%)	19 (27%)	6 (9%)	8 (12%)

The types of comedones found are listed in Table 5. The commonest variety of comedone was the microcomedone, with the closed type accounting for only 9% of the total. A microcomedone was one in which the duct showed minimal but definite distension and the granular layer in the ductal epithelium was prominent.

Fourteen biopsies were stained with the monoclonal antibody battery and in all of them the pan T cell marker stained the majority of the lymphoid cells in the inflammatory infiltrate. In nine of the biopsies it was possible to calculate the helper : suppressor T lymphocyte ratio in the infiltrate, the average value being 2·8 : 1. Dendritic cells staining for OKT6 were seen in the dermal infiltrate in many biopsies.

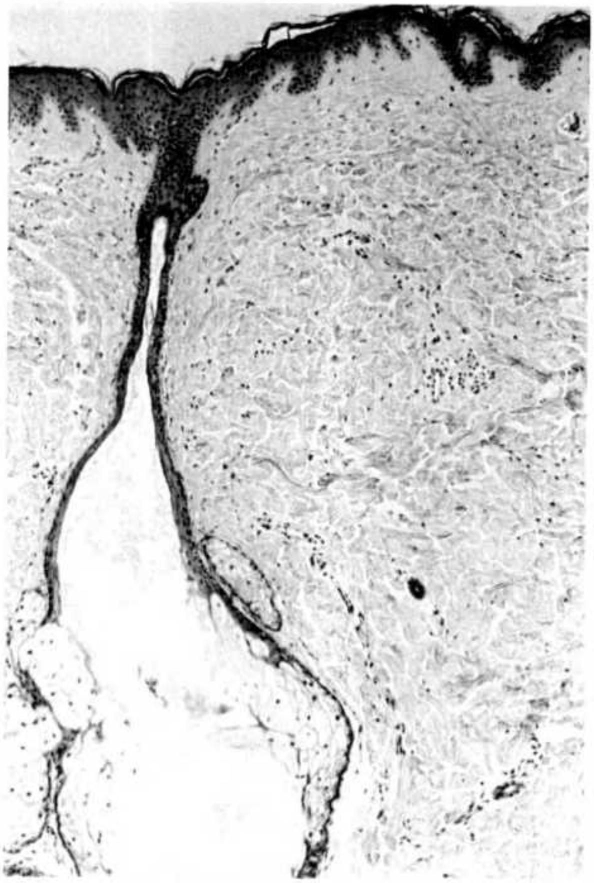
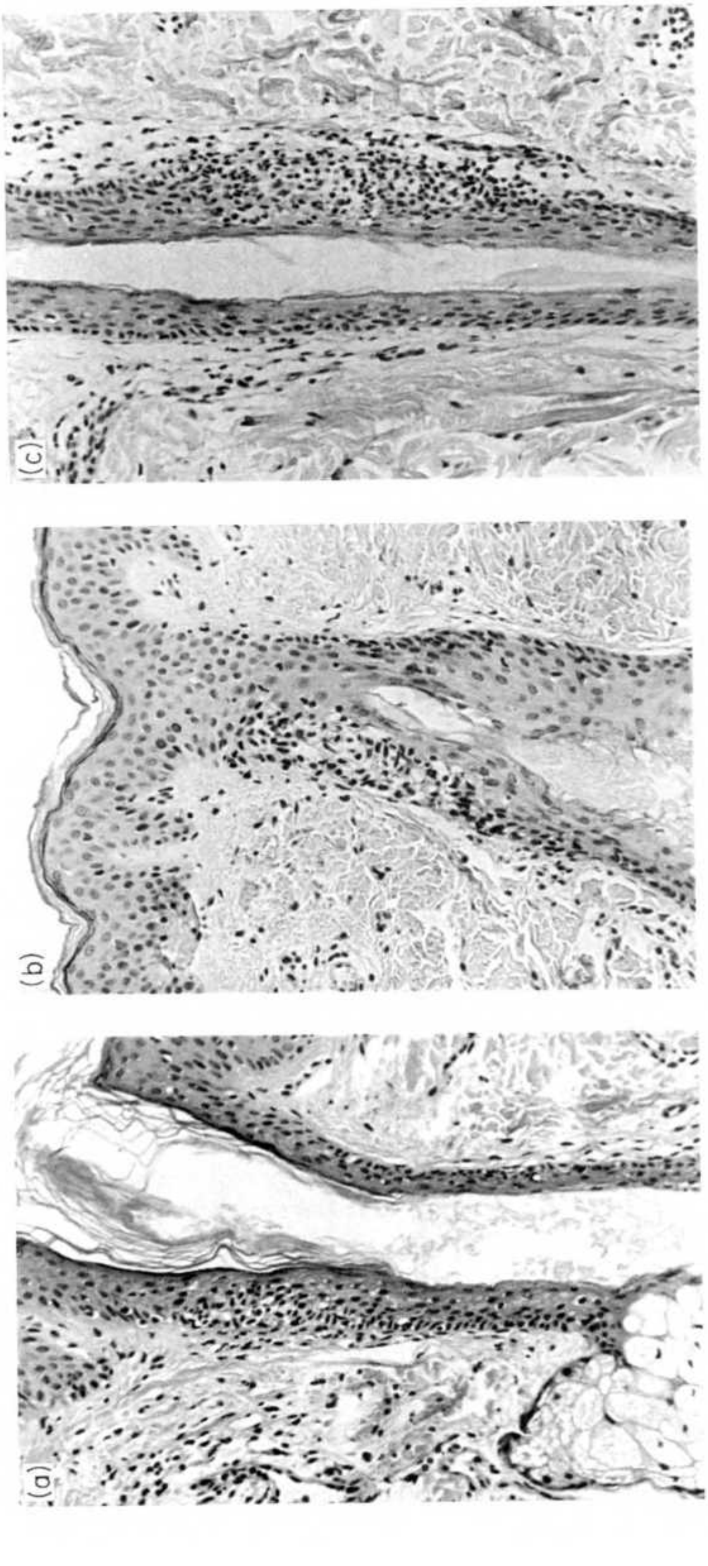


FIGURE 1. Minimal perivascular infiltrate with no cellular activity around the duct or gland.



FIGURES 2 a, b and c. Increasing degrees of spongiosis and lymphoid infiltration of the duct wall. The spongiosis contrasts with the normal epithelium on the opposite wall.

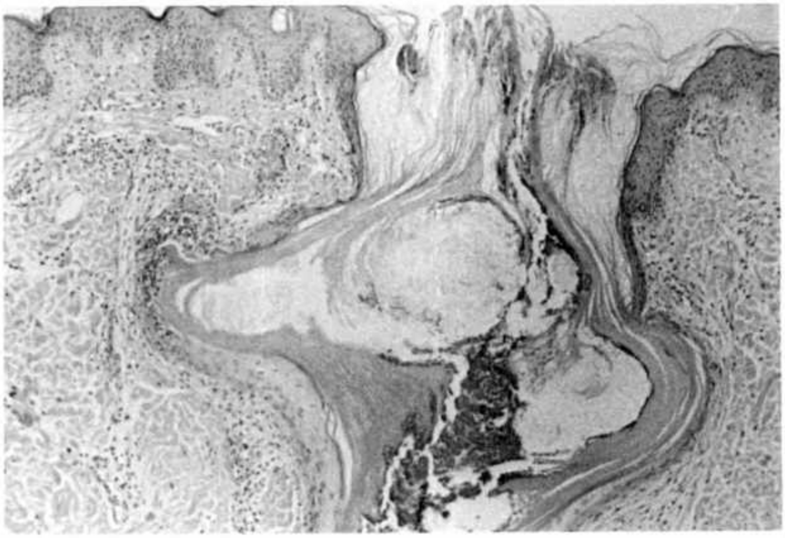


FIGURE 3. Duct rupture, centre left, due to pressure from within the duct.

There was an overlap in the inflammatory findings at 6, 24 and 72 h; although there was a trend towards increasing cellular activity with time, it was always possible to find an 'active' gland at 6 h, and a quiescent gland at 72 h. It was however possible to construct a composite picture of the inflammatory scenario in an acne lesion from the earliest changes through to duct rupture.

The earliest change was a minimal lymphoid perivascular infiltrate accompanying a minor degree of vascular dilatation, but with no activity around the gland or duct (Fig. 1).

The next stage was local spongiosis of the duct wall with a gradually increasing lymphoid infiltrate and disruption of the duct wall (Figs. 2a,b and c). With the development of spongiosis, and generally not earlier, PMNs began to appear; they were initially seen intravascularly, and then appeared within the duct itself. At this stage they were not seen in any great numbers in the periductal or general infiltrate.

The PMNs then accumulated in increasing numbers within the duct, frequently distending it to form a pustule. A combination of continuing spongiosis and duct distention by PMNs then led to a localized rupture. Two of the biopsies, however, showed a different type of rupture which appeared to be due to pressure from within the distended lumen; the epithelial wall was very attenuated and ruptured at the point where the comedonal material was bulging most. In contrast to the 'inflammatory' ruptures described above, which demonstrated duct wall spongiosis and a lymphoid infiltrate, these 'mechanical' ruptures showed no evidence of spongiosis, and the associated infiltrate consisted of PMNs which accumulated outside, rather than inside, the duct (Fig. 3).

DISCUSSION

Previous studies of the infiltrate in acne vulgaris have differed in their findings. Lynch,[12] Strauss *et al.*[13] and Vasarinsh[14] all described the lymphocyte as the initial cell in the inflamed lesion, whilst Kligman[1] opined that the first appearance was made by the PMN; it is Kligman's view that seems to hold sway today. One problem with earlier studies was that biopsies were taken of lesions which, because they were small, were thought to be early; this, however, cannot be assumed to be the case. Our method of using an acrylic sheet allowed us to distinguish three groups of early lesions: 0–6 h old, 0–24 h old, and 48–72 h old.

We know from our previous studies that a follicle showing surrounding erythema will develop into an acne lesion,[15] such a follicle, therefore, should contain the earliest evidence of the acne inflammatory process.

We were initially surprised at how many biopsies had very little cellular activity. Although all but two of the biopsies had some degree of perivascular infiltration, there were 12 (6 at 6 h, 5 at 24 h and 1 at 72 h) which showed this alone with no sign of focal or generalized activity around the duct or lobules and no evidence of disruption of the duct.

A perivascular infiltrate is often present in non-lesional skin of acne patients (J.F.B.Norris, unpublished observation) and thus some of the perivascular infiltrate found in our biopsies may be unrelated to the inflammatory process.

Following on from these minimal perivascular changes there was a wide range of immunological events: spongiosis in the duct wall, focal and generalised infiltrates around the duct, infiltrates within the duct and duct rupture. In general, lymphoid cells outnumbered PMNs in the 6 h biopsies and PMNs became increasingly numerous in the 24 h and 72 h lesions. It was, however, possible to see a very 'active' gland with a PMN infiltrate at 6 h, and equally a very quiescent gland at 72 h with a lymphoid infiltrate. This suggests that individual lesions can become inflamed at different rates—some slowly and some rapidly. Thus, although the present data has been analysed chronologically, an alternative approach would be to redefine early and late lesions in terms of the histological findings.

One way of doing this would be to make the not unreasonable assumption that those biopsies without duct disruption (spongiosis or rupture) are behaving as biologically early lesions, whilst those with duct disruption are behaving as late lesions. Thus there were 42 late lesions with duct disruption and 27 early ones with intact ducts.

When the infiltrates in these two groups were re-examined, the non-disrupted (early) lesions had a lymphoid infiltrate in 85% of cases and a PMN infiltrate in 15%; whilst the disrupted (late) lesions had a lymphoid infiltrate in 28% of cases and a PMN infiltrate in 72%.

It appears, therefore, that whether the biopsies are analysed in a strictly chronological fashion or with regard to duct disruption, the conclusion is the same: that the lymphoid cell is the predominant early cell and that the PMN arrives later.

The most useful aspect of the monoclonal antibody studies was to show that the lymphoid cell infiltrate was predominantly lymphocytic. The fact that the T helper/suppressor ratio was 2·8 : 1 is probably non-specific as similar ratios are found in many other inflammatory dermatoses.

The exception to this lymphocyte preponderance was found in the two biopsies showing 'mechanical rupture' in which PMNs, and not lymphocytes, were found in association with the corneocytic material that was liberated from within the duct. This perhaps helps to explain the finding by Dalziel[16] of a PMN infiltrate in response to injected corneocytes, but it would appear from the present work that a predominantly PMN infiltrate is not typical of early acne inflammation.

The early lymphocyte infiltrate is concentrated mainly perivascularly, but can occur both focally and generally around the duct. The PMN usually arrives after the duct has been disrupted and then appears to be strongly attracted into the ductal lumen; a PMN infiltrate was found in 31 of the 42 disrupted lesions and in 28 of these it was confined primarily within the ductal lumen. In the 27 lesions with intact ducts, only one had a PMN infiltrate.

The prominence of the lymphocyte in these early biopsies may suggest the production of lymphotactic factors within the duct or the ductal epithelium. The overall picture, however, of a perivascular lymphocytic infiltrate and epithelial spongiosis with lymphocytic aggregation is very similar to the cell mediated delayed hypersensitivity reaction seen in allergic contact dermatitis. We suggest that the inflammatory process in the acne lesion may be initiated by such a delayed hypersensitivity reaction.

The possibility of an immunological mechanism in acne has been considered previously; lymphocyte transformation studies have been positive in the presence of *P. acnes*, but there was no evidence of macrophage migration inhibition,[6] although others have found this latter test to be positive in subjects with more severe forms of acne.[17] Delayed skin test reactivity to *P. acnes* gives indirect support for the presence of cell-mediated immunity.[7] Although *P. acnes*, or one of its integral antigens such as cell wall carbohydrate,[5] is the most likely target of such a delayed hypersensitivity reaction, other constituents of the duct, such as keratin, can perhaps also be considered as possible antigens. Keratin does stimulate circulating antibodies,[18] although when injected intradermally it appears to produce primarily an irritant response.[16] There are, however, many small molecular weight keratin breakdown products within the duct which potentially may act as allergens.[19]

The similarity between the histological findings in the early acne lesion and those in allergic contact dermatitis leads us to the hypothesis that the patient with acne develops a 'contact sensitivity' to an antigen—*P. acnes* or a keratin breakdown product—within his own sebaceous ducts. When the antigen concentration reaches a certain level a cell mediated immune reaction is triggered. PMNs are then attracted to the site by chemotactic agents which are either generated during the reaction or have been exposed within the ductal lumen by damage to the duct wall.

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The effect of simple warming procedures on finger blood flow in systemic sclerosis

M.J.D.GOODFIELD, A.HUME AND N.R.ROWELL

Department of Dermatology, General Infirmary at Leeds, Great George Street, Leeds LS1 3EX, U.K.

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SUMMARY

Finger blood flow was measured by laser Doppler flowmetry in 15 patients with Raynaud's phenomenon (RP) due to systemic sclerosis (SS), and 15 normal controls. Measurements were performed in a temperature controlled room at 28°C. The blood flow in the patients was significantly lower than in the controls ($P < 0{\cdot}001$). After hand warming in water at 35°C for 10 min, blood flow in the patients and controls did not differ significantly.

Following this, the response to a standardized cold stress produced similar falls in both groups to levels that were not significantly different and these occurred over a similar time course. After cold stress ended, there was recovery of blood flow in both groups, and blood flow after 20 min was not significantly different between the two groups.

The induced vasodilatation persisted, in those patients in whom it was remeasured, for at least 2 h. Repeating the experiments at a room temperature of 24°C produced similar results.

This indicates that considerable vasodilatation is possible in these patients, and can be produced by simple means. It also indicates that local and central thermoregulatory reflexes are intact. Cold induced symptoms in patients with SS are related to low resting blood flow, not to cold sensitivity, and simple warming may provide a useful treatment.

The majority of patients with systemic sclerosis (SS) suffer from Raynaud's phenomenon (RP) at some stage of their disease. A small number develop frank ischaemic lesions of their extremities.[1] The histological nature of the vascular problem has been well described,[2] and is characterized by endothelial damage, basement membrane changes and sub-intimal sclerosis.[3] The consequences of this process may be easily visible in the nail folds, where capillary dilatation and loss are said by some to be a marker for more severe disease,[4] although this is not confirmed by more recent work.[5,6]

The vascular damage produces a reduced resting peripheral blood flow in SS patients which has been confirmed by all the techniques that have been applied to measure it—xenon washout studies,[7] strain plethysmography,[8] and laser Doppler flowmetry.[9] Cold challenge reduces blood flow further, and may stop it altogether if Raynaud's phenomenon is induced.[10] It is difficult,

Correspondence: Dr M.Goodfield.

however, to measure reductions in flow below an already very low baseline. It is known that vasodilator drugs given intravenously improve symptoms,[11] and periods of intensive warming are also effective in reducing Raynaud's phenomenon (N.R.Rowell, unpublished data). The effect of locally increased temperature on peripheral blood flow has been little investigated, however,[12] and we have examined this using laser Doppler flowmetry in patients with SS and Raynaud's phenomenon.

METHODS

Fifteen patients with SS who had RP and 15 age- and sex-matched normal controls were investigated. All non-essential drugs and all vasodilating agents were stopped for one week before the investigations. Cigarettes were discontinued 24 h before the investigations (although only one patient and none of the controls were smokers).

Investigations were performed after 30 min equilibration in a constant temperature room at 28°C. Resting blood flow was measured using a laser Doppler flowmeter (Perimed PF1b) attached to the finger tip of the third finger on the left hand, unless for anatomical reasons this was not possible. Both hands were then immersed in water in a water bath at 35°C for 10 min, after which finger blood flow was recorded for a further 10 min before a standard cold stress was applied.[13] This consisted of air blown at a constant rate through a box containing ice and thence onto the left hand. The temperature of the air was 18–19°C, and the stress of cold air was applied for 10 min, finger flow being monitored continuously. This stress was sufficient to induce symptoms in all patients and some of the controls, and produced blood flow changes in all subjects. After cold stress, finger blood flow was monitored for a further 20 min. In five patients, blood flow was remeasured after an interval of 2 h, during which time the patients had been allowed to carry out normal activities inside or outside the hospital.

Resting flow, maximal flow after warming, minimum flow and time to minimum after cold stress, maximum flow after recovery from cold stress and the time to achieve this were all recorded, as was the flow 2 h later in five patients. Statistical analysis was carried out using Wilcoxon's signed rank test.

In a further five patients with SS, the studies were repeated at normal room temperature.

RESULTS

The patients were directly matched for age and sex with the controls (Table 1) and had a wide range of disease extent as shown by disease scores calculated by the method of Hughes *et al.*[14] Figure 1 shows the resting blood flow values in each group, confirming the considerable reduction in the patients with SS (median 0·2 V, range 0·1–1·0 V) compared with the controls (median 3·2 V, range 0·9–6·0 V) ($P < 0{\cdot}01$). After 10 min warming, the increase in finger flow was considerably greater in the patients, resulting in laser Doppler blood flow values that were not significantly different between the two groups (median 3·5 V, range 0·5–7·0 V and median 4·2, range 1·0–6·5 V, respectively) (Fig. 2). The reduction in flow that occurred after application of the cold stress is shown in Figure 3, (post-cold stress values: median 2·8 V range 0·1–6·0 V and 3·1 V range 0·2–5·0, respectively). This was similar in the two groups; that is, patients with SS responded normally to experimental cold stress after simple warming. Furthermore, the extent and time course of recovery after cold stress was also similar in both groups (median 2·2 V range 0·2–6·3 and 2·8 range 0·6–5·5, respectively) (Fig. 4).

TABLE 1. Details of patients and control subjects who took part in the study

	Patients	Controls
Age (years) (mean and range)	49 (19–70)	48 (20–70)
Sex	12 F, 3 M	12 F, 3 M
Duration of disease (years) (median and range)	5(2–28)	...
Disease score[14] (median and range)	8(2–11)	...

In the five patients in whom blood flow was measured 2 h later, the vasodilatation induced by simple warming appeared to have persisted to some extent (Fig. 5).

Figure 6 shows the increase in blood flow after hand warming in the 5 patients in whom the experiments were repeated at an environmental temperature of 24°C. There was, once again, a considerable increase in laser Doppler flow.

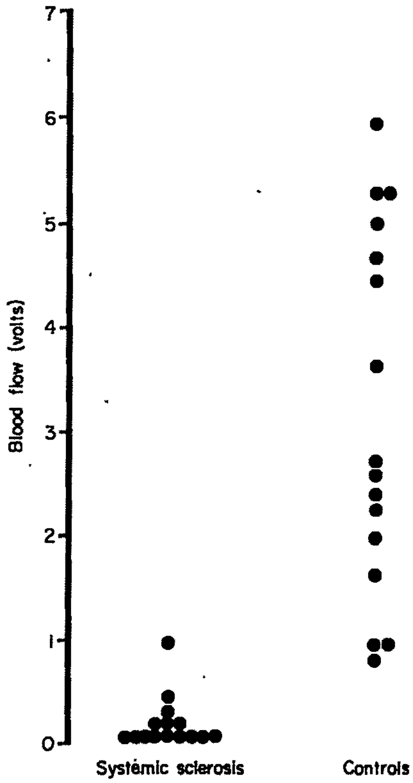

FIGURE 1. Resting blood flow in the patients and the controls ($P < 0.01$).

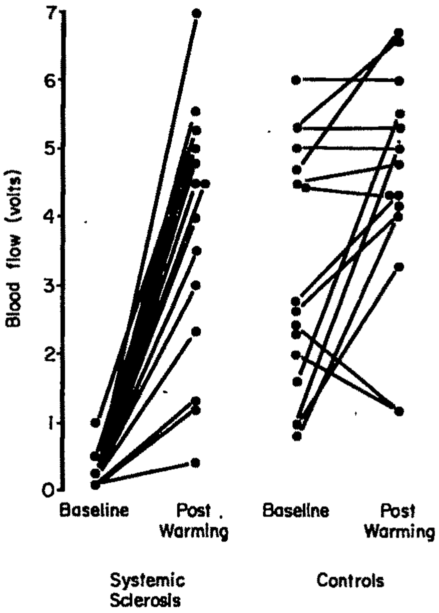

FIGURE 2. Response to warming in the patients and controls. Blood flow after warming was not significantly different in the two groups ($P > 0{\cdot}1$).

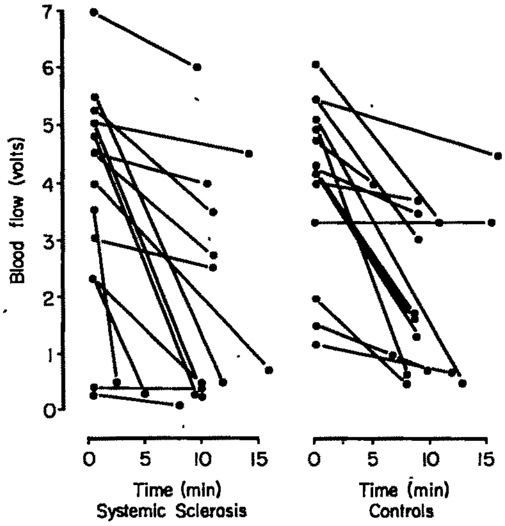

FIGURE 3. Response to cold stress in patients and controls. Fall in blood flow in the two groups was not significantly different ($P > 0{\cdot}1$) and the time courses were similar ($P > 0{\cdot}1$).

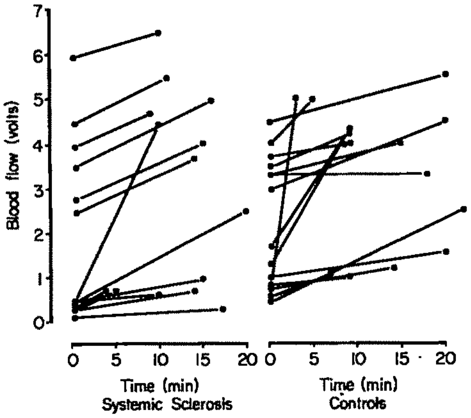

FIGURE 4. Passive rewarming in the patients and controls. The extent and time course were similar in both groups ($P > 0.1$).

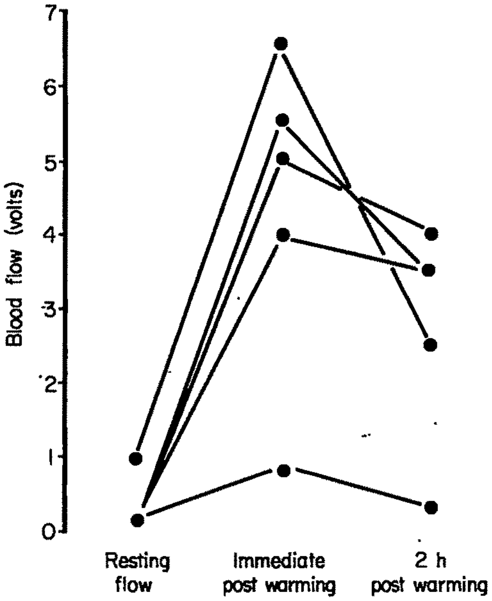

FIGURE 5. Blood flow before and immediately after warming and 2 h later in five patients with SS.

DISCUSSION

The nature of the small vessel abnormality in Raynaud's phenomenon is not known. Raynaud's original idea that it was produced by a central defect of sympathetic control,[15] and Lewis' theory of a local vascular abnormality[16] remain unconfirmed. It is suggested that in primary RP the defect may be one of central sympathetic control,[17] whilst in secondary RP in SS a structural defect in peripheral vessels may be responsible,[18] suggesting a more fixed abnormality.

The findings in the present study do not support this latter hypothesis, since a combination of

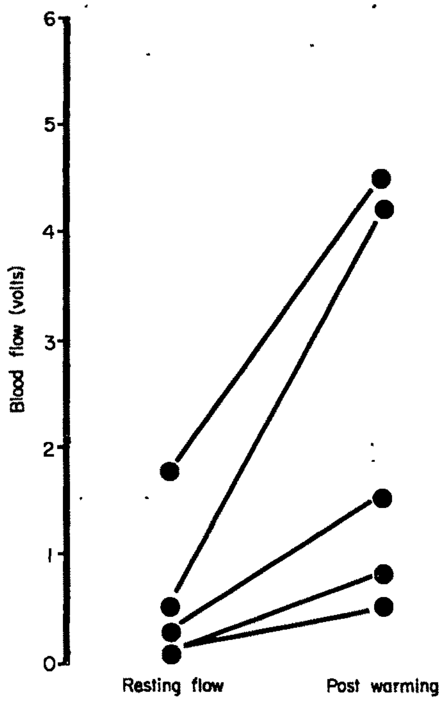

FIGURE 6. Effect on blood flow of warming the hands at room temperature (24°C) in five patients with SS.

whole body (ambient temperature 28°C) and local warming, produced finger blood flow values that were not significantly different from the normal controls. Local warming alone was sufficient to induce vasodilatation in the five patients in whom this was measured. This indicates considerable vasodilatation, even in those patients with the lowest resting blood flow. The response to subsequent cold stress was similar to that in the controls, both in terms of fall in flow and in the time course and extent of recovery. Previous studies, using xenon washout techniques without initial warming, have also indicated proportionately similar results for SS patients and controls,[9] but it is important that by the simple warming procedure more direct comparison can be made between patients with SS and others without such impairment in resting blood flow. The warmed hand appears to be a useful starting point for comparative investigations.

The resting vasoconstriction is not fixed therefore; whatever the vessels may look like histologically, they are able to behave normally once the resting vasoconstriction is relieved. Furthermore, even after the application of a cold stress, there is good recovery of flow. This may explain why digital gangrene is a relatively rare event in patients with SS, despite the severity of their vascular symptoms. Whether nutritional capillary blood flow rather than arteriovenous shunt flow is increased cannot be determined by laser Doppler flowmetery.

The thermoregulatory response to warming has two components: a centrally mediated autonomic reflex, responding to changes in core temperature, and a direct local effect of heat on vessel tone.[19] In our study we have made use of both of these components, although it seems to be the effect of local warming that is the major factor inducing the changes in flow, since hand warming at normal room temperature produced similar changes. The study does suggest that both this autonomic reflex and the local response to increased temperature are intact in patients with SS.

In addition, the study indicates the considerable variation in the response to cold within the normal control group (Fig. 3), despite the absence of symptoms in these subjects. The degree of vascular cold responsiveness is clearly not the only factor in the development of RP, and other factors such as tissue conductivity may be important. Temperature change is known to influence the viscosity of connective tissue surrounding blood vessels, limiting the extent to which blood vessels can dilate.[20] However, our preliminary investigation of simultaneous changes in skin temperature during these experiments suggests that this falls profoundly during cold stress, and takes some time to recover. It is unlikely that connective tissue viscosity influences the recovery in blood flow to any great extent, although of course, viscosity changes in the blood may do so.

From a practical viewpoint, since symptoms of RP in SS must be due to the low resting blood flow, this can be increased by the simple methods used here. Patients should be advised to warm their hands thoroughly before planned cold exposure. This should also warm the soft tissues of the hands.

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Effect of nicotinamide on the phototest reaction in polymorphous light eruption

B.ORTEL, DANIELA WECHDORN, A.TANEW AND H.HÖNIGSMANN

Division of Photobiology, Department of Dermatology I, University of Vienna, Austria

Accepted for publication 26 November 1987

SUMMARY

The effect of nicotinamide on the phototest response was investigated in 14 patients suffering from polymorphous light eruption (PMLE). In contrast to the favourable effect in the prevention of PMLE reported by other groups, oral nicotinamide did not affect the phototesting results in our patients.

Apart from sunburn, polymorphous light eruption (PMLE) is the most common photodermatosis. It affects predominantly younger adults and may reach a prevalence of 10% among Caucasians.[1] The aetiology and pathogenesis are still unknown. The variability in the history and clinical appearance indicate that PMLE may comprise a pathogenetically heterogenous group of sun sensitivity disorders. The diagnosis of PMLE is based on history, clinical examination and the result of phototesting.[2] Exclusion of antinuclear antibodies in the serum is mandatory.

The reproduction of skin lesions by artificial light sources is a valuable diagnostic tool[3] and action spectra have been established by several groups.[2,4–7] In most patients the action spectrum of their PMLE lies in the longwave ultraviolet (UVA, 320–400 nm) range. A smaller percentage is sensitive to shortwave UV (UVB, 280–320 nm) or to both UVA and UVB. In a few patients sensitivity to visible light has also been demonstrated.[4,7] The success rate for reproduction of skin lesions under artificial conditions varies with exposure schedule, season and choice of the appropriate test area.[2,6,8]

Dermatologists have the choice of several treatment options, but none is perfect for all cases. The highest efficacy is documented for phototherapy with UVB and for psoralen photochemotherapy.[6,7,9] Topical sunscreens are of benefit in some cases,[10] especially those absorbing in the UVA range,[2] but only in less sensitive patients. As compared with phototherapeutic measures, which are time consuming and not available everywhere, oral medication appears to be more convenient. Beta-carotene[11–14] and antimalarial drugs[13–16] may provide some protection, the latter however carry the risk of potential ocular toxicity. A recent study[17] suggests that oral nicotinamide may be effective in preventing PMLE. The rationale for the use

Correspondence: Bernhard Ortel, Division of Photobiology, Department of Dermatology I, University of Vienna Medical School, Alserstrasse 4, A-1090 Vienna, Austria.

of systemic nicotinamide is based on theoretical considerations, on tryptophan metabolism[18] and on uncontrolled clinical observations.

In order to examine this phenomenon we have compared the phototest reaction of PMLE patients before and during nicotinamide treatment under controlled conditions.

METHODS

Patients

Three male and 11 female patients, mean age 35 years (range 21 to 46 years) were enrolled in the study. The diagnosis of PMLE was established by history and a positive phototest result. The mean duration of the disorder was 10·5 years (range 3 to 22 years). Screening for antinuclear antibodies was negative in all patients and no other medications were taken during the study.

Radiation sources

Phototesting with UVA was performed with a high intensity UVA source (Sellas Sunlight 2001, Sellas Medizinische Geräte GMBH, Gevelsberg-Vogelsang, West Germany) equipped with a metal halogen lamp and a 5 mm thick window glass filter. This unit emits a broad peak at 350–400 nm with an output of 60 mW/cm^2 at a distance of 15 cm, as measured with an IL 600 International Light photometer and a UVA detector with peak sensitivity at 360 nm. The UVB emission of this source is less than 0·01%. Phototesting with UVB was performed with a solar simulating metal halogen lamp (Sellasol, Sellas Medizinische Geräte GMBH, Gevelsberg-Vogelsang, West Germany) emitting 0·85 mW/cm^2 as measured with an IL 600 International Light photometer and a UVB detector with peak sensitivity at 297 nm, at a distance of 30 cm.

Phototest procedure

In all patients the minimal erythema dose (MED) was established on the untanned buttock skin, for UVA and UVB, respectively. Subsequently two areas of 16 cm^2 (4 cm × 4 cm) were outlined with a template at a site previously affected by PMLE. One area was exposed to UVB, and the other to UVA, daily on successive days until PMLE lesions developed. Initial irradiation consisted of 80% of the MED, and 20% increments were made according to erythemal response. This procedure was repeated during nicotinamide treatment, at the contralateral site. The test areas were evaluated 24 h after each exposure. The appearance of lesions in the test areas typical of the individual patient's rash was considered as a positive test result.

Medication

Oral nicotinamide (Nicovitol®, 500 mg tablets, Lannacher Heilmittel G.M.B.H., Styria, Austria) was administered for 1 week prior to retesting, at a daily dose of 3 g, administered in three 1 g doses, and this was continued throughout the second test period.

RESULTS

Before nicotinamide treatment, nine out of 14 patients had a positive test result in the UVA irradiated area, two had lesions induced by UVB and the remaining three developed PMLE lesions in both test areas. The lesions appeared after one to six exposures (mean 3·5). Detailed results are shown in Table 1.

On Nicovitol® treatment none of the patients experienced an aggravation of the phototest reaction. In eight patients no change in skin sensitivity was observed. Three patients had a

TABLE 1. Results of phototesting before and during treatment with nicotinamide in 14 patients with PMLE

							Last exposure before positive reading					
					PMLE induced by		Before nicotinamide			During nicotinamide		
Patient no.	Age (years)	Sex	Skin type	Test site	UVA	UVB	No. of exposures	UVA (J/cm^2)	UVB (mJ/cm^2)	No. of exposures	UVA (J/cm^2)	UVB (mJ/cm^2)
1	22	F	II	upper arm	+	–	6	100	—	6	100	—
2	23	F	II	V-shaped area of the neck	+	–	4	80	—	2	60	—
3	43	F	III	upper arm	–	+	6	—	60	5	—	40
4	46	M	I	upper back	+	+	1	—	10	1	—	10
				upper back			3	40	—	2	30	—
5	46	M	II	V-shaped area of the neck	–	+	5	—	70	5	—	70
6	31	F	II	thigh	+	–	3	80	—	4	80	—
7	44	F	I	upper arm	+	+	3	70	—	4	80	—
				upper arm			3	—	40	4	—	40
8	45	M	II	forearm	+	–	4	80	—	4	80	—
9	21	F	III	upper arm	+	–	4	90	—	4	90	—
10	44	F	I	breast	+	–	3	80	—	3	80	—
11	36	F	II	upper arm	+	+	3	90	—	3	90	—
				upper arm			3	—	50	3	—	50
12	26	F	III	upper arm	+	–	3	80	—	3	80	—
13	31	F	II	forearm	+	–	3	80	—	3	80	—
14	25	F	III	forearm	+	–	2	90	—	3	100	—
Mean	35						3·5	80	46	3·5	80	42

decrease in sensitivity by one step, that means one more exposure was needed to provoke lesions during Nicovitol® treatment. The remaining three patients showed an increased sensitivity; two of them showed lesions 1 day earlier than at the first testing, another had a two step increase in sensitivity.

DISCUSSION

Evaluation of protection against PMLE has to face several challenges. Many patients may suffer from their sun sensitivity under a variety of natural conditions. One group of PMLE patients, however, will develop the rash only under certain circumstances, for example, while sunbathing in a southern climate without preceding sun exposures. PMLE usually tends to aggravate over the years and can cease to appear spontaneously after several years. Placebo treatment has been shown to be successful in a relatively high percentage of patients.[13,14] In order to address these issues, cumulative sun exposure has been measured by actinometers; personal recordings and questionnaires have supplied valuable information for comparison of the varying conditions inducing PMLE.[13] Large groups of patients and inclusion of placebo groups make PMLE studies more reliable.

Phototesting is well established as a diagnostic method for PMLE. Because of the nature of the present study only patients with a clearly readable positive test result were enrolled. Comparison of phototest reactions before and after therapy has been demonstrated as a sensitive procedure to assess the value of preventive treatment with sunscreens[2,19] and PUVA.[20]

The rationale for the use of nicotinamide to protect against PMLE was based on uncontrolled clinical observations and single case reports. One recent open study showed good results with oral nicotinamide at daily doses of 3 g with respect to the clinical course. In a preliminary trial we could not reproduce these findings. Thus we decided to carry out an open study using patients with clearly visible positive test results. Since the preliminary findings had not demonstrated protection by nicotinamide we did not include a placebo control group. As we were unaware of the duration of any protection nicotinamide might provide, the phototest performed without the drug had to be carried out first. This made a blind trial design impossible.

The clinical details of the individuals taking part in the present study were similar to those reported in larger groups of PMLE patients and our subjects can thus be considered to be representative.

A broad variety of phototesting procedures is in use in different countries and institutions. The number of exposures and the wavelengths may differ considerably as does the method of reading the results.[6] Whilst employing our schedule, a change in the phototest response can be indicated by a larger number of exposures or by a larger cumulative dose required to elicit skin lesions. However, a change of one 'step' does not appear to represent a very great alteration in skin sensitivity and the only subject in whom there was a two step change in the number of exposures, experienced increased sensitivity to the test irradiation during nicotinamide treatment.

These results indicate that nicotinamide does not prevent the development of PMLE lesions induced by artificial UV sources and it does not significantly change the exposures required, or the action spectrum of the disease.

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Successful resolution of severe perianal ulceration with photochemotherapy (PUVA)

F.CARABOTT, R.M.VALORI* W.R.BURNHAM,† R.D.R.CAMP,
J.E.LENNARD-JONES* AND C.BLAIR†

St John's Hospital, London, *St Mark's Hospital, London, and †Oldchurch Hospital, Romford, Essex U.K.

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SUMMARY

We report a case of recurrent perianal ulceration associated with gastrointestinal symptoms, but without evidence of underlying disease, that responded to a course of photochemotherapy (PUVA).

CASE REPORT

A 54-year-old heterosexual man presented in 1973 with a 2-year history of muddy loose motions, two to three times daily. Examination was normal apart from a perianal abscess, which improved with antibiotics. In 1980 he presented with another perianal abscess and inguinal lymphadenopathy. Examination under anaesthetic revealed two sinus tracts near the anal verge, but no fistula formation. The sinus tracts were laid open, but with little improvement. Histopathological changes of both inguinal lymph node and granulation tissue were non-specific. In 1982 the following investigations were normal or negative: full blood counts, blood viscosity, biochemical profile, thyroid function, autoantibody screen, syphilis serology, Kveim test, Mantoux test, stool examination for ova and parasites, gastroscopy, duodenal biopsy, small bowel enema, sigmoidoscopy and colonoscopy. Colonic biopsies showed a non-specific, moderate increase in chronic inflammatory cells. Perianal skin biopsies showed non-specific chronic inflammatory changes with no granuloma formation. Treatment with disodium cromoglycate, clofazimine and various antibiotics produced no improvement.

Between 1982 and 1984, he had episodes of painful fissuring and ulceration on average once a month. Oral prednisolone (40 mg daily) and sulphasalazine did not influence the bowel habit, stool consistency or perianal lesions. In 1985 he was treated with dressings soaked in prednisolone-21-phosphate solution with definite, but incomplete, improvement. Examination at that stage showed extensive perianal ulceration and fissuring (Fig. 1a). Repeated investigations for underlying disease were negative, and bacteriological examination of a perianal swab showed normal flora. Perianal skin biopsy showed a non-specific diffuse infiltrate of lymphocytes and plasma cells in the upper dermis.

This case was presented at a clinical meeting of the St John's Hospital Dermatological Society, 3 December 1987.

Correspondence: Dr F.Carabott, Research Registrar, St John's Hospital, London WC2H 7BJ, U.K.

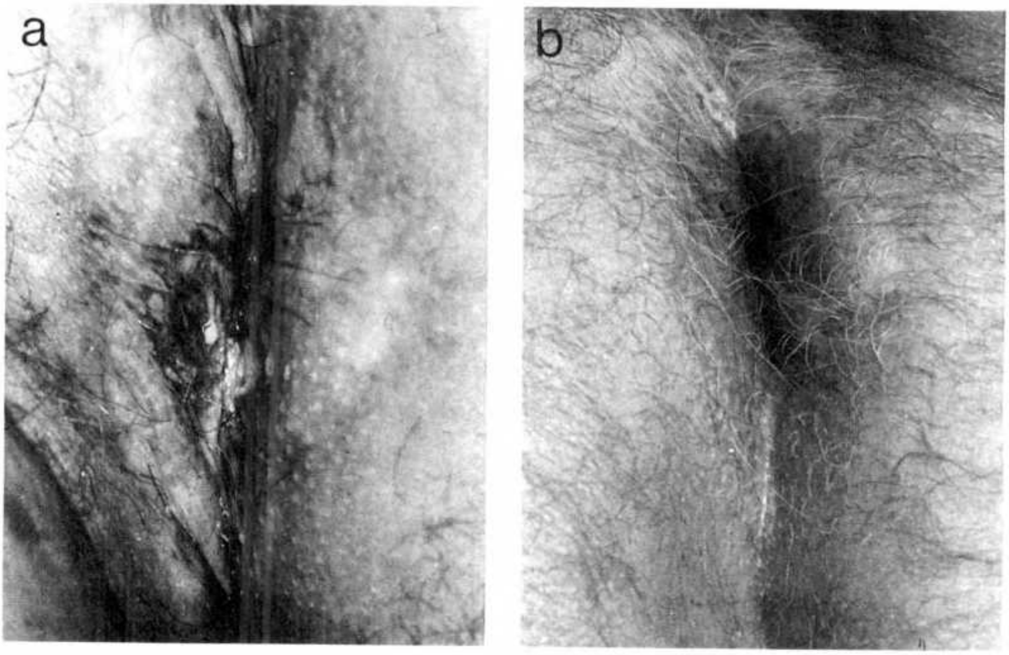

FIGURE 1. The perianal area, (a) prior to starting PUVA, (b) after 10 weeks of PUVA therapy.

A trial of PUVA to the perianal area was started in November 1985. Treatment was given thrice weekly using 40 mg of 8-methoxypsoralen orally 2 h before irradiation with high intensity ultraviolet A (UVA) radiation from a Waldmann UV800 unit. The patient lay semi-prone and exposed the area by splaying the buttocks with a gloved hand. UVA exposure was started at 0·5 J/cm^2 with weekly increments of 0·5–1·0 J/cm^2 depending on the UVA tolerance of the irradiated area. After 10 weeks the patient was able to sit comfortably for the first time in 5 years. Examination showed complete healing (Fig. 1b). At this point the UVA dose stood at 9 J/cm^2 with a cumulative UVA dose of 150 J/cm.^{2} Treatment frequency was then reduced to twice weekly for 10 weeks (UVA exposure dose 11 J/cm^2) and was then reduced further to once weekly for 7 weeks (UVA exposure dose 11 J/cm^2). At this point the patient developed an exacerbation with perianal tenderness and swelling, but without ulceration or fissuring. PUVA frequency was increased to twice weekly (UVA exposure dose 11 J/cm^2) for 4 weeks with resolution of signs and symptoms. The PUVA frequency was once again reduced to once weekly (UVA exposure dose 12 J/cm^2) for 28 weeks with no further recurrence of symptoms. Since March 1987 (32 weeks at the time of writing) the patient has remained asymptomatic on PUVA once fortnightly with a gradually decreasing UVA dose to 9 J/cm^2, despite no change in bowel habit or stool consistency. The patient did not at any stage develop any painful erythema of the irradiated area.

The patient's quality of life improved considerably; he could walk and sit without discomfort.

DISCUSSION

Perianal ulceration may be associated with Crohn's disease, Behcet's disease, and infections such as syphilis and tuberculosis,[1] but in our patient no underlying disease was found. Perianal ulceration is a distressing condition for which medical therapy offers incomplete or temporary palliation, and surgery is rarely beneficial.[1] PUVA therapy of our patient's refractory ulceration resulted in sustained remission, and a minor relapse appeared to respond promptly to a temporary increase in treatment. PUVA has been shown to be effective in a number of skin conditions, chiefly psoriasis, vitiligo and mycosis fungoides,[2] but its use for perianal inflammation with ulceration has not been reported previously. The rationale for PUVA treatment in our patient is that PUVA is known to exert an anti-inflammatory action, possibly through its effect on T lymphocytes. Erythemogenic doses of PUVA in normal subjects have been shown to produce a reduction in circulating T cells with an increase in the proportion of null cells.[3] Long-term PUVA treatment of psoriatic patients is associated with a reduction in circulating helper/inducer T cells.[4] PUVA has also been shown to decrease the blastogenic response of peripheral mononuclear cells to phytohaemagglutinin (PHA) stimulation.[5] PUVA has been shown to suppress T cell locomotion in response to both chemotactic and chemokinetic factors.[6] Exposure of lymphocytes to PUVA has been found to increase chromosomal aberrations and the frequency of sister-chromatid exchanges.[7]

Although PUVA is a relatively new treatment and the long-term skin cancer risk has not been fully evaluated, the benefit in our patient appears to justify its continued use. Studies of the role of PUVA in other forms of perianal disease, particularly related to Crohn's disease, now appear warranted.

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The action spectrum in quinine photosensitivity

B.L.DIFFEY, P.M.FARR* AND S.J.ADAMS†

Regional Medical Physics Department, Dryburn Hospital, Durham, *Department of Dermatology, Royal Victoria Infirmary, Newcastle and †Department of Dermatology, Carter Bequest Hospital, Middlesbrough, U.K.

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SUMMARY

The action spectrum for 24-h ultraviolet erythema was determined in three elderly patients who presented with a photosensitive eruption and who were taking quinine sulphate for night cramps. In each case the *in vivo* action spectrum was consistent with the absorption spectrum of quinine, and extended from about 370 nm to at least the lower wavelength limit of terrestrial sunlight (approximately 300 nm). Calculations based upon the combination of the action spectrum for quinine photosensitivity, the spectrum of terrestrial sunlight, and the transmission properties of various topical sunscreens, indicated that a broad absorption spectrum sunscreen would be required to provide adequate photoprotection.

Quinine is commonly used to treat nocturnal cramps. Topical contact with quinine has been reported to cause both contact[1] and photocontact[2] allergy. Systemic treatment with quinine sulphate can result in an eczematous type of photosensitivity with increased erythemal sensitivity to UVA radiation,[3] or in lichen planus in a photosensitive distribution.[4]

The only *in vivo* phototesting reported, to our knowledge, in systemic quinine photosensitivity has been limited to broad band UVB and UVA sources. We report here the results of action spectrum studies on three photosensitive patients whilst taking, and after discontinuing, quinine sulphate, and compare the *in vivo* action spectra with the *in vitro* absorption spectrum of quinine.

METHODS

Absorption spectrum of quinine

Quinine sulphate was dissolved in phosphate buffered saline (PBS) (pH 7·4) at a concentration of 1 mg/ml. An appropriate volume was transferred to a quartz cuvette and the absorption spectrum measured in the wavelength interval 280 to 420 nm using a spectrophotometer (Pye Unicam SP8-150). A 2 mm path length cuvette was used for measurements between 280 and 350

Correspondence: Dr B.L.Diffey, Regional Medical Physics Department, Dryburn Hospital, Durham DH1 5TW, U.K.

nm, and a 10 mm path length cuvette for measurements at longer wavelengths, to compensate for the rapidly decreasing extinction with increasing wavelength. A cuvette of the appropriate path length containing PBS was placed in the reference beam.

Subjects

Three patients (one male, aged 69 years and two females aged 73 and 84 years) were studied. All had, a chronic, dusky erythematous, scaling eruption that was considered clinically to be photosensitive because of the sharply demarcated localization of the rash to light exposed sites. Sun exposure caused acute worsening of the rash with oedema and eczematous changes (Fig. 1). The duration of the rash before diagnosis varied from 4 months to 2 years. In addition to quinine sulphate, taken regularly for night cramps, all patients were receiving other medication (Table 1). The time of onset of symptoms after starting quinine sulphate treatment was about 4 weeks in two of the patients, and resolution occurred in all cases within 8 weeks of stopping treatment. In each case the rash resolved following withdrawal of quinine sulphate but with no change in the other medication.

Phototesting

The apparatus used consisted of a 900 W xenon arc lamp optically coupled to a single grating monochromator as described in detail elsewhere.[5] A liquid-filled light guide transmitted the radiation from the exit slit of the monochromator to the patients' skin, resulting in an irradiation field 5 mm in diameter. An optical glass filter (Schott type WG335) was inserted into the distal end of the light guide for irradiation at wavelengths above 330 nm to eliminate any stray UVB radiation. For each spectral region examined, radiant exposures were increased geometrically by a ratio of $\sqrt{2}$, thus doubling alternate exposures. Irradiance was measured using a thermopile. Phototesting was carried out on the back on skin of normal clinical appearance. The irradiation sites were examined 24 h following exposure and the dose of radiation in each of the spectral regions noted that produced a just perceptible reddening of the skin. Phototesting was performed on two occasions: after a period of at least 2 weeks treatment with quinine sulphate and approximately 3 months after stopping quinine.

RESULTS

Absorption spectrum of quinine

The absorption spectrum of quinine sulphate is shown in Figure 2. The compound exhibited maximal absorption at 330 nm.

Action spectra studies

The minimal phototoxic doses (MPD) determined whilst the patients were taking quinine and the minimal erythemal doses (MED) determined after discontinuing therapy are listed in Table 2.

The relative effectiveness of radiation of different wavelengths in causing quinine-induced photosensitivity is expressed by the action spectrum, $\sigma(\lambda)$. In defining the action spectrum we assumed that erythema induced by the quinine is independent of the photochemical events leading to ultraviolet-induced erythema via normal pathways, and that erythema produced by these two routes is linearly additive.[6] Consequently at wavelength λ nm:

$$\sigma(\lambda) = 1/\text{MPD} - 1/\text{MED} \qquad \text{(Equation 1)}$$

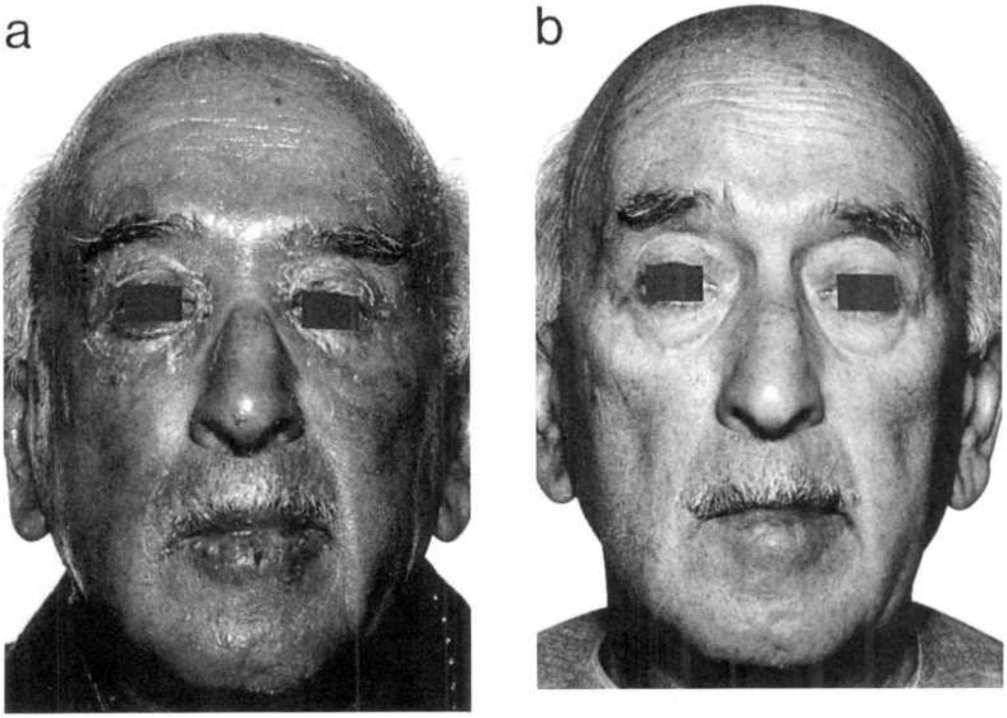

FIGURE 1. (a) The photosensitive eruption in Patient 3 whilst taking quinine. (b) Appearance 3 months after discontinuing treatment with quinine.

TABLE 1. Medication being taken by the three patients in addition to quinine sulphate

Patient no.	Medication
1	Aldactide (spironolactone and hydroflumethazide), oxprenolol, dipyridamole
2	Salbutamol, hydroflumethazide, temazepam
3	Frusemide, Moduretic (amiloride and hydrochlorothiazide), allopurinol, digoxin

In order to compare the action spectrum with the absorption spectrum of quinine, it was necessary to express the MPDs and MEDs in 'photochemical units' of quanta/cm^2 rather than in radiometric units (J/cm^2). A dose of radiation measured in quanta/cm^2 is simply proportional to the product of the dose measured in J/cm^2 and the wavelength of the radiation (Planck's law).

The action spectrum for quinine photosensitivity for each of the three patients is shown in Figure 3. The broken curve in Figure 3 is the expected action spectrum for quinine

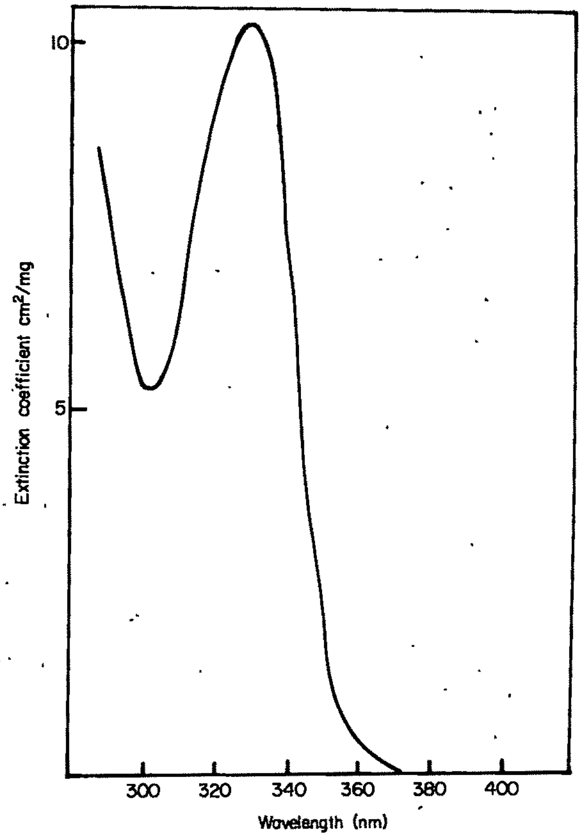

FIGURE 2. The extinction coefficient of quinine sulphate in phosphate buffered saline.

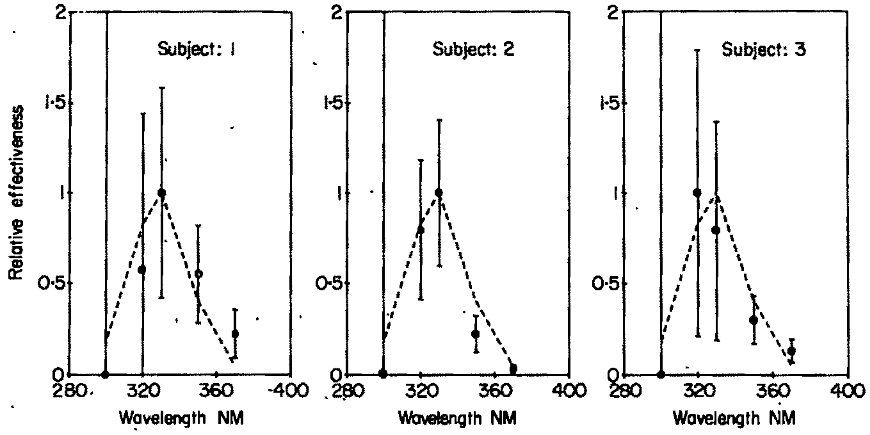

FIGURE 3. The observed *in vivo* action spectrum (Equation 1) for quinine photosensitivity (points) in each of the three patients, and the expected action spectrum derived from the *in vitro* absorption spectrum of quinine (– – –) normalized to unity at 330 nm. The error bars represent the uncertainty of the observed action spectrum.

TABLE 2. Minimal phototoxic doses (MPD) and minimal erythema doses (MED) observed in each of the three patients, and the 95% confidence interval of MEDs in unsensitized, normal skin. The doses are expressed in J/cm^2

Central wavelength ± bandwidth (nm)		Patient 1	Patient 2	Patient 3	Normal range
300 ± 5	MPD	0·028	0·056	0·028	0·015–0·060
	MED	0·028	0·056	0·028	
320 ± 10	MPD	2·0	0·70	0·50	1–4
	MED	2·8	2·8	1·0	
330 ± 15	MPD	2·5	0·63	0·63	2·8–11
	MED	7·0	5·0	1·3	
350 ± 30	MPD	5·0	2·5	2·5	14–56
	MED	20	14	14	
370 ± 30	MPD	10	10	5·0	20–80
	MED	28	20	20	

photosensitivity assuming the site of action is at the basal layer of the epidermis. This curve was calculated from consideration of the absorption spectrum of quinine (Fig. 2), the transmittance of UVR through the epidermis[7] and the spectral power incident on the patient for each central wavelength/bandwidth combination on the irradiation monochromator.[6]

DISCUSSION

The suspected clinical diagnosis of photosensitivity was confirmed by monochromator testing in each patient. The abnormal erythema action spectrum, with sensitivity confined principally to the UVA region, suggested drug-induced photosensitivity and the results of repeat monochromator testing performed after withdrawal of quinine confirmed that this drug was responsible in each case. This must be an infrequent complication of quinine treatment as this drug is widely used for the treatment of night cramps and yet very few cases of associated photosensitivity have been reported.

It may be seen from Figure 3 that, for each patient, the expected action spectrum, based on the *in vitro* absorption spectrum of quinine, is consistent with the observed *in vivo* action spectrum defined by Equation 1. The large error bar at 300 nm is a consequence of error analysis applied to Equation 1[6] and is consistent with the common observation that for a variety of photosensitizing drugs the MED and MPD to wavelengths in the UVB and UVC regions are normally the same. The reason for this is illustrated by the following example. From Table 2 it can be seen that at 330 nm (the peak of the absorption spectrum for quinine), the MED increased from 0·63 to 5 J/cm^2 when Patient 2 discontinued therapy. We can calculate that the radiation dose expected to cause a minimal erythema from the drug alone would be the reciprocal of 1/0·63–1/5, that is 0·72 J/cm^2. From the broken curves in Figure 3 it can be seen that the expected relative effectiveness of 300 nm radiation is about one fifth of that at 330 nm. In other words, the equivalent dose at 300 nm due to the drug alone would be 0·72 × 5 = 3·6 J/cm^2. If this value is combined with the MED of 56 mJ/cm^2 in the unsensitised skin of Patient 2 we would expect that when she was taking the drug, her MPD at 300 nm would fall to 55 mJ/cm^2—a reduction of only 2% from the

TABLE 3. Approximate exposure periods around the middle of the day outdoors in the U.K. required for quinine-photosensitized subjects who are shopping, walking etc to receive one minimal phototoxic dose

Topical photoprotection	Season		
	Winter	Spring/autumn	Summer
None	4 h to all day	1 h	0·5 h
UVB sunscreen e.g. Spectraban 15	all day	2 h	1·5 h
UVA+B sunscreen e.g. Coppertone Supershade RoC total sunblock	all day	all day	6 h

MED in her unsensitized skin. This small change would be impossible to detect in practice. In fact, a drug would need to exhibit very high absorption in the UVB, coupled with a low MPD to UVA radiation, for drug-induced UVB photosensitivity to be reliably demonstrated *in vivo*.

Inspection of Table 2 indicates that Patient 3 continued to exhibit increased erythemal sensitivity at 320 and 330 nm after discontinuing quinine therapy. This is probably due to the fact that throughout the period of study he was taking a diuretic containing hydrochlorothiazide (Table 1). Although Patients 1 and 2 were also taking a thiazide diuretic (hydroflumethazide), hydrochlorothiazide is almost certainly a more potent photosensitizer than hydroflumethazide. In the period 1981–86 there were 54 reports of photosensitivity attributed to hydrochlorothiazide, and only one attributed to hydroflumethazide (Committee on Safety of Medicines, personal communication). Also, in a recent study of thiazide-induced photosensitivity,[8] 23 out of 33 patients investigated were taking hydrochlorothiazide (Moduretic or Dyazide), yet none were referred with photosensitivity induced by hydroflumethazide.

Determination of the action spectrum for drug-induced photosensitivity can provide other useful information apart from confirmatory evidence about the cause of the photosensitivity. The data can be used to estimate the effectiveness of various topical photoprotective agents against sunlight exposure. The technique involves combining estimates of the spectral irradiance of terrestrial sunlight at different times of day and seasons[9] with the minimal phototoxic doses at various wavelengths for quinine photosensitivity, and the transmittance of solar ultraviolet radiation through a topically-applied sunscreen.[10] The mathematical expressions are integrated over both wavelength and period of exposure such that the time required for a subject outdoors, who may be shopping or gardening, to receive sufficient exposure to result in a minimal phototoxic response is determined.

Calculations were carried out for exposure during different seasons and for a subject applying either no sunscreen, a sunscreen effective just for UVB (e.g. Spectraban 15) or a broad attenuation spectrum sunscreen (e.g. Coppertone Supershade or RoC total sunblock). The results are summarized in Table 3 where it may be seen that the minimal phototoxic dose may be exceeded following 30 min–1 h exposure to sunshine during spring, summer or autumn, and even following several hours of bright winter sunshine. The use of a UVB-effective sunscreen, such as Spectraban 15, offers only minimal photoprotection. This is not unexpected since the action spectrum for quinine photosensitivity extends to longer wavelengths than the absorption spectrum of this type of sunscreen. On the other hand, a broad attenuation spectrum sunscreen, such as Coppertone Supershade or RoC total sunblock, should provide good photoprotection throughout the whole year providing the sunscreen is applied at the recommended surface density of 2 mg/cm^2. Indoor activities (apart from exposure to artificial sources of UVR) should not give rise to photosensitive reactions. However, patients should be advised not to sit for long periods near a window, as window glass will transmit ultraviolet radiation at wavelengths down to about 310 nm, that is, well into the action spectrum for quinine photosensitization.

Since this paper was accepted for publication a similar study of quinine photosensitivity has been published.[11]

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Tolerance of spironolactone

BRONWYN R.HUGHES AND W.J.CUNLIFFE

Department of Dermatology, The General Infirmary, Leeds U.K.

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SUMMARY

A survey of 54 patients taking spironolactone for hirsutes or acne showed that side-effects occurred in 91%; in 80% of patients, these were related to the anti-androgenic mechanism of the drug (menstrual disturbances, and breast enlargement and tenderness). The concomitant use of a contraceptive pill gave a lower incidence of menstrual abnormalities. Only seven patients (13%) had to stop the drug. In a further eight patients, a reduction in dose to between 125 and 175 mg daily achieved a compromise of controlling the disease and the side-effects. Side-effects tended to occur early and so regular review during the initial 3 months of treatment is advised.

Two patients in our study developed 'chloasma'—a previously unreported complication of spironolactone. This was the only side-effect which occurred late in treatment.

Spironolactone was first noted to have anti-androgenic properties by Corvol.[1] Since then, spironolactone has been used successfully to treat hirsutism and acne. It is thought to exert its anti-androgenic effect by suppression of androgen production by gonads by blocking the enzyme cytochrome P450, and by competitive inhibition of dihydrotestosterone (DHT) at the skin androgen receptor.[2]

Spironolactone is known to have a number of side-effects, the commonest of which are gynaecomastia and impotence in males, and menstrual disturbances in females. Previous workers have claimed minimal side-effects when reporting studies of the efficacy of spironolactone in the treatment of hirsutism with doses up to 200 mg per day.[3,4] Most of these studies have reported small numbers of patients who have been treated for short periods of time. We have investigated the incidence of side-effects in our patients to see if the apparent paucity of side-effects was due to the tolerance of a population of younger patients.

Spironolactone has been shown to have an effect on sexual differentiation in foetal male rats[5] (abnormalities in the development of the urethra and prostate). Because of the likelihood of an effect on a male foetus, particularly at 12–16 weeks gestation, female patients of childbearing age should be prescribed this drug only under adequate contraceptive cover. Tremblay[2] suggested that the addition of an oral contraceptive to the regimen reduces the incidence of side-effects. We have studied this in more detail.

Correspondence: Dr B.R.Hughes, Department of Dermatology, The General Infirmary, Leeds LS1 3EX, U.K.

METHODS

All patients who had been prescribed spironolactone for acne or hirsutes in our clinic were contacted. Those still attending the clinic were interviewed. All patients who had been treated previously, but were no longer attending the follow-up clinic and were not willing to attend, were asked to fill in and return a questionnaire listing possible side-effects with time of onset and duration of symptoms. The questions asked related to all side effects documented in previous studies and by the Committee on Safety of Medicines.

The dose of the drug, the duration of treatment, and any reduction in dosage were recorded. The method of contraception was noted and, for the oral contraceptive pill, the time of initial prescription prior to starting treatment with spironolactone was recorded.

RESULTS

Fifty four patients, (53 female, one male) mean age 34 years, (range 21–51 years) were included in the analysis. Thirty eight patients were interviewed and 16 returned a questionnaire. The indications for treatment were hirsutes (24 patients), acne (21 patients), both acne and hirsutes (eight patients) and seborrhoea (one patient). Six patients had undergone hysterectomy; these and the male patient were not included in the analysis of menstral disturbances.

All patients were initially prescribed spironolactone 200 mg/day. The mean duration of treatment was 15 months (range 1–45 months). Twenty three patients were taking the oral contraceptive pill.

Only five patients (9%) experienced no side-effects. Disturbance of menstruation was the commonest side effect (72% of patients). The incidence of other side effects is shown in Figure 1.

The gynaecological side effects in 23 patients on the pill were compared with those in 24 patients not on the pill (Fig. 2). All the patients on the oral contraceptive pill had been taking this preparation for at least 6 months prior to starting spironolactone. The analysis excluded the patients who had undergone hysterectomy and the male patient. The number of patients on the pill who experienced side-effects was less than the number among patients using other methods of contraception; the proportions of patients with gynaecological problems were 88% and 52%, respectively.

Thirty nine per cent of patients admitted to having a dry skin on treatment; five of these patients reported this as being beneficial, i.e. skin greasiness returning to normal. The remaining patients felt their skin had become excessively dry—six of the patients with acne (28%) and 11 of those with hirsutes (45%). Five of these patients complained, in addition, of rashes, with scaling and itching of the skin. This was relieved by reducing the dose of spironolactone or the addition of an emollient.

Two patients developed a 'chloasma'-like pigmentation of the face. A 35-year-old woman treated for both acne and hirsutes, returned to the clinic complaining of increasing pigmentation on the cheeks and forehead after 8 months of treatment. On examination there was patchy hyperpigmentation on the forehead, lower cheeks and chin. Because of the beneficial effects of the drug she was reluctant to stop treatment and the hyperpigmentation persisted. The second patient was a 30-year-old woman who had been on spironolactone for her acne for 12 months. While on holiday in Spain she had noticed pigmentation on her forehead and around her eyes. Spironolactone was stopped and the pigmentation gradually faded. Neither patient was on the

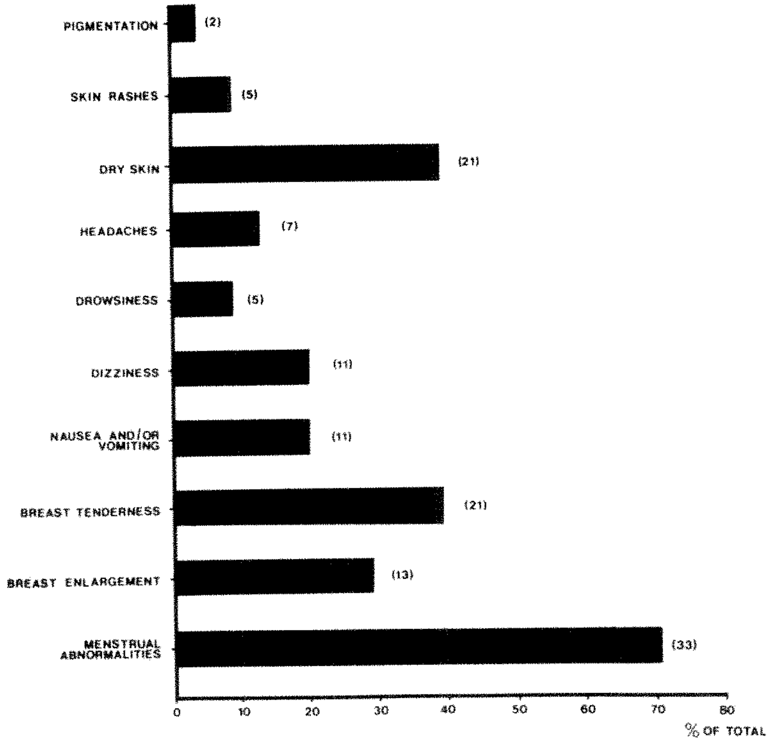

FIGURE 1. Side-effects in 54 patients taking spironolactone. Numbers in parentheses are numbers of patients.

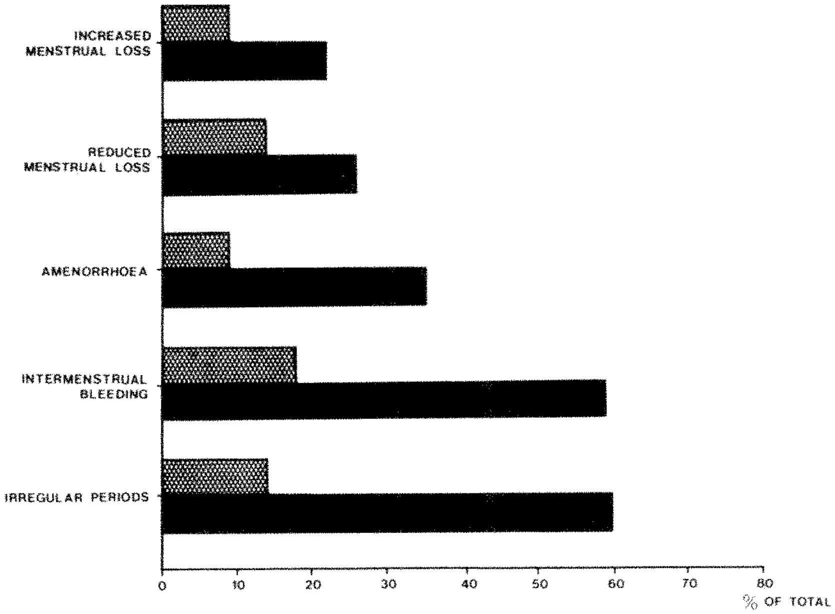

FIGURE 2. Menstrual abnormalities in 47 patients taking spironolactone; 23 patients were taking the oral contraceptive pill (▨) and 24 were using other methods of contraception (■).

oral contraceptive pill, or had been recently pregnant, nor had there been previous pigmentation. Both patients were on 200 mg spironolactone daily.

Only seven patients (13%) withdrew from the treatment. The reasons for cessation of the drug were nausea (two patients), persistent vaginal bleeding (two patients) postural hypotension (one patient) and chloasma (one patient). One patient withdrew from treatment because of a desire to become pregnant. A further eight (15%) required reduction in the dosage for various reasons—increased menstrual loss or irregular periods or both (six patients), amenorrhoea (one patient) and skin dryness (one patient). This reduction controlled the side-effects, but in three patients not the disease. Therefore, as a compromise a dose between 125 and 175 mg was given to control the disease and minimize the side-effects.

DISCUSSION

Previously studies have quoted an incidence of side-effects ranging from 0–75%.[2–4,6,7] Tremblay[2] suggested that side-effects could be kept at a minimum level of 3–5%, but in his studies low doses of 50 to 70 mg of spironolactone were used, which in our patients were found to be ineffective. Other studies in the treatment of hirsutism quote minimal side effects of transient diuresis only[3] and diuresis and oligomenorrhoea in two patients out of 39.[4] In the two studies in which spironolactone was used for the treatment of acne, side-effects, in the form of menstrual disturbances and transient diuresis were noted in seven of 12 patients,[7] and in 11 of 15 patients [6] menstrual irregularity, nausea, dizziness, skin dryness and breast swelling occurred. In these studies doses of up to 200 mg/day of spironolactone were used. Other reports do not comment on side-effects.

We found a high incidence of side-effects which tended to persist throughout treatment. However, in most cases, the patients were prepared to tolerate the side-effects in view of the beneficial effects of the drug. The side-effects, with the exception of facial hyperpigmentation and skin dryness and rashes occurred almost immediately after starting treatment, i.e. within the first month. The patients with acne experienced an improvement within the first month in that their skin felt less greasy, and the number of acne lesions decreased within the first 3 months. However, the patients with hirsutes did not improve until after 4 months and so required motivation to persist despite side-effects. Five of the patients who withdrew from treatment within 4 weeks, had done so before experiencing any beneficial effects of the drug and felt the side-effects were too severe to continue.

Some patients, particularly those with menstrual abnormalities, found that these symptoms settled without alteration of the dose, but not until after three months of treatment. We were able to relieve the cutaneous side-effects in all but one of our patients by the use of emollients.

Pregnancy is contra-indicated whilst on spironolactone because of the risk of feminization of a male foetus. All patients must be warned of this possibility. However, spironolactone can be given with the contraceptive pill and these patients had fewer gynaecological side effects. Fifty per cent of our patients were aged 30 years or more and most had completed their family. Spironolactone is particularly suitable for patients in whom an oestrogen/cyproterone acetate combination is contra-indicated.

AUTHORS' NOTE

Since this paper was accepted for publication, readers will be aware of the statement which appeared in the Committee on Safety of Medicines' Current Problems (January 1988) that potential human metabolic products of spironolactone are carcinogenic in rodents.

We and other parties are looking into the precise significance of these findings and, for the present, not commencing any patients on spironolactone as a new therapy.

Searle, the major manufacturers of spironolactone, state that the safety of the drug has been confirmed by epidemiological studies in man and that no cases of carcinogenesis can be related to the use of this drug in 25 years of clinical use. However, the company intends to start further prospective studies to confirm the safety record.

We wish to remind readers that in the British National Formulary it is stated that cyproterone acetate has produced hepatic tumours in animals and so, as with this drug, the risk/benefit ratio must be carefully considered.

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High dose nicotinamide in the treatment of necrobiosis lipoidica

SUSAN HANDFIELD-JONES, S.JONES AND R.PEACHEY

Department of Dermatology, Bristol Royal Infirmary, Bristol BS2 8HW, U.K.

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SUMMARY

An open study of high dose nicotinamide in the treatment of 15 patients with necrobiosis lipoidica is reported. Of 13 patients who remained on treatment for more than 1 month, eight improved. Improvement took the form of a decrease in pain and soreness, a decrease in erythema and the healing of ulcers if present, although the skin did not return completely to normal in any patient. There were no significant side-effects, particularly with respect to diabetic control, an important finding as lesions tended to relapse if treatment was stopped.

Necrobiosis lipoidica is a granulomatous condition which usually arises on the shins, although lesions have been reported to occur on other parts of the body. It is usually found in association with diabetes (although only a small proportion of diabetic patients develop this lesion) and the pathogenesis is unknown. However, abnormalities in dermal collagen,[1] vascular supply of the skin,[2] and immunological responses[3,4] have been demonstrated.

The lesions, which comprise yellow, telangiectatic, atrophic plaques with a variable degree of erythema usually only present a cosmetic problem, although in some cases they can be painful or itchy, or may ulcerate. Available treatment, including topical steroids, systemic aspirin and dipyridamole, and surgical excision and grafting tend to be unsatisfactory. Following the report of the use of high dose nicotinamide in granuloma annulare,[5] we report the results of an open study of high dose nicotinamide in patients with necrobiosis lipoidica and present an illustrative case history.

CASE REPORT

A 25-year-old lady presented in 1985 with a 6-year history of abnormal ulcerated skin on the right lower leg. Insulin dependent diabetes mellitus had been diagnosed in 1974 and was controlled on twice daily Actrapid® and Monotard® insulins, totalling 72 units per day.

On examination there was a 6 × 5 cm yellow, telangiectatic, atrophic area consistant with necrobiosis lipoidica, containing two ulcerated areas, 3 mm and 10 mm in diameter (Fig. 1). She was started on nicotinamide 500 mg t.d.s. and when seen two months later there had been a marked improvement in the soreness, with a significant decrease in erythema. Ten weeks later the ulcerated areas had healed completely (Fig. 2). The nicotinamide was, therefore, stopped and within 3 days the patient noticed increasing redness and soreness. She was, therefore,

Correspondence: Dr S.Jones.

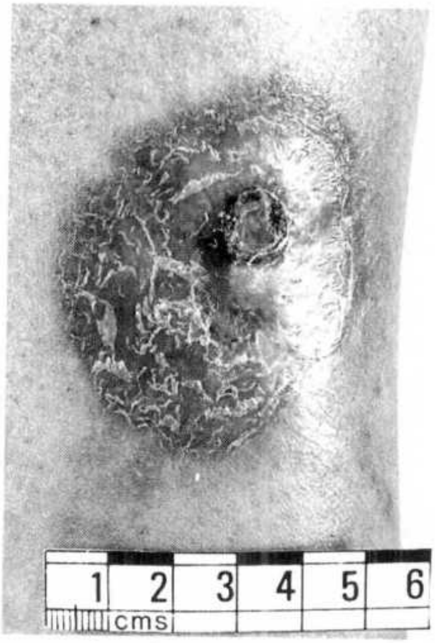

FIGURE 1. Area of necrobiosis prior to nicotinamide therapy showing ulcerated areas.

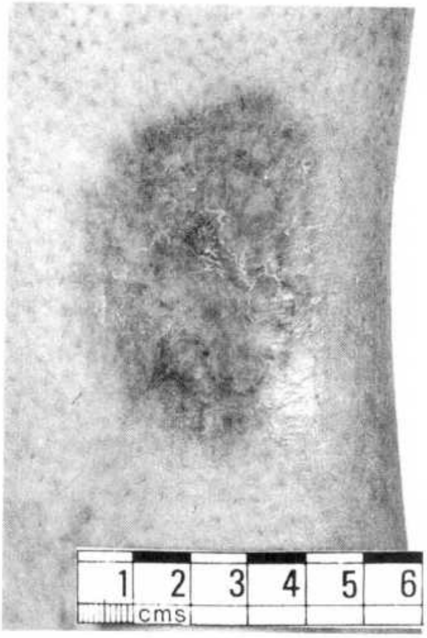

FIGURE 2. Area of necrobiosis after nicotinamide therapy showing healing of ulcerated areas.

restarted on nicotinamide 1·5 g per day and after 2 weeks the lesion had paled and become comfortable. Subsequent reductions of the dosage to 1 g daily resulted in an increase in symptoms, and therefore the patient remained on 1·5 g daily.

Diabetic control was monitored carefully whilst on therapy because of the reported diabetogenic effect of nicotinic acid.[6] Contrary to expectations, diabetic control improved and the patient was able to reduce her dose of insulin from 72 to 55 units daily, whilst glycosolated haemoglobin levels fell from 11·9 prior to therapy to 9·4 whilst on 1·5 g of nicotinamide daily. On stopping treatment, insulin requirements rose to pre-treatment levels within a few days, and fell again when treatment was re-instituted. Liver function tests remained normal throughout treatment.

RESULTS OF CLINICAL TRIAL

Fourteen other cases of necrobiosis lipoidica were treated with nicotinamide and details of these are given in Table 1 (the patient described above was Patient 1). In two patients, treatment was stopped prematurely; in Patient 4 because of a recurrence of atrial fibrillation probably unrelated to therapy, and in Patient 6 because of non-specific malaise. Thus there were 13 patients in whom the response to treatment could be evaluated.

In all patients, at least 1 month's therapy was required before any improvement was seen and Patients 2, 3, 5, and 9 all took between 2 and 3 months to respond. The two patients with pain at the site of the lesions both noted marked pain relief and Patient 5 also reported improvement of painful diabetic neuropathy. Six patients showed reduction in inflammation with lesions becoming paler, and Patient 8 developed areas of normal skin within the lesions. Four patients, including Patient 1 had long-standing ulceration. In three, the ulcers healed completely and in the other the ulcers decreased in size (as assessed by serial measurement of the widest diameter).

TABLE 1. Details of 15 patients with necrobiosis lipoidica treated with nicotinamide

Patient no.	Sex	Age (years)	Diabetes*	Lesions: Site	Lesions: Duration (years)	Lesions: Symptoms	Result of treatment
1	F	27	IDDM	Shin	6	Ulcer 6 × 5 cm, pain	Ulcer healed, pain free
2	F	67	MODM	Legs/arms	7	—	Paler, flatter, some cleared
3	M	58	IDDM	Shins/arms	6	—	Improved—1 g no effect
4	F	63	—	Shins	1	—	Stopped in 1st month
5	F	44	IDDM	Legs/hand	?	Pain	Less pain, paler
6	F	20	IDDM	Legs/arms	2	—	Stopped after 2 weeks
7	F	34	IDDM	Shin	15	—	No change
8	F	34	IDDM	Widespread	10	—	Paler
9	M	37	IDDM	Shin	20	Multiple erosions	Erosions healed, paler
10	F	25	IDDM	Shin	5	Ulcer 3 × 2·5 cm	Ulcer smaller, paler
11	F	38	—	Shin	2	—	No change
12	F	44	IDDM	Shin	1	—	No change
13	F	25	IDDM	Shin	1	—	Extended
14	F	57	—	Leg/head	1	—	No change
15	F	25	IDDM	Shins	10	Ulcer 4 × 3 cm	Ulcer healed

* IDDM—insulin dependent diabetes mellitus
MODM—maturity onset diabetes mellitus

Of the eight patients who improved, five remained on treatment. Patients 2, 9 and 15 stopped treatment and had not relapsed after follow-up periods of 2 years, 8 months and 10 months, respectively. Patients 1 and 10 relapsed on decreasing the dose. Patient 1 improved again when the dose was increased, but Patient 10 had not improved after 2 months further therapy. The other three patients remained on therapy, improvement was maintained and they were free from side-effects. In no patient was there complete clearing of lesions and it would seem that nicotinamide does not affect the long-standing changes in dermal collagen seen in these lesions.

DISCUSSION

Nicotinamide is the amide of nicotinic acid (niacin, vitamin B_3), and it forms part of the key co-enzyme, NAD (nicotinamide adenine dinucleotide) which acts as a hydrogen ion acceptor in oxidation/reduction reactions. It has been shown *in vitro* to suppress lymphocyte transformation in response to a number of mitogens and antigens.[7]

In the past, high dose nicotinamide has been used to treat a number of dermatological conditions, including erythema multiforme,[8] dermatitis herpetiformis,[9] erythema elevatum diutinum,[10] polymorphic light eruption,[11] erythema induratum[12] and, recently, granuloma annulare.[5] Its mode of action in the latter condition was postulated to be via the inhibition of release of lymphokines, this leading to less active macrophage migration. As necrobiosis lipoidica and granuloma annulare are very similar histologically, both being granulomatous conditions of unknown aetiology, it might be expected that this drug would have a similarly beneficial effect in necrobiosis. The present study was conducted with this postulate in mind.

Nicotinic acid and nicotinamide are not identical in their actions and side-effects. Nicotinic acid tends to have a number of side-effects including a vasodilatory flush,[13] nausea and vomiting[6] and a variable effect on diabetes, some studies showing an improvement in glucose tolerance, whilst most clinical studies show a deterioration of diabetic control.[6,14] It has also been shown to increase glycogenolysis and gluconeogenesis, in addition to having an anti-lipolytic effect.[14] Nicotinamide on the other hand seems to have fewer side-effects, flushing being seen less commonly with this drug.[13] In addition, its effect on diabetic control seems to be less marked; a large study of high dose nicotinamide in the treatment of schizophrenic patients by Hoffer[6] noted no change in carbohydrate metabolism, although only one diabetic patient was included. Indeed, there is only one report of a single diabetic patient whose control deteriorated on treatment with nicotinamide and stabilized again on cessation of therapy.[15] Hepatotoxicity has been described as a rare complication of high dose nicotinamide therapy.[6] It would seem however, that nicotinamide, even in high dosage, is relatively free from these side-effects, especially if given as 500 mg tablets rather than the standard 50 mg tablets.[6] No side-effects were noted in our series of patients.

We suggest, therefore, that nicotinamide can be a useful therapy for some patients with necrobiosis lipoidica, although a double-blind trial is obviously needed to confirm this. Patients most likely to benefit are those with pain or soreness associated with marked inflammatory change or ulceration. The long-standing changes in dermal collagen are unlikely to respond to therapy at this dosage, although it would be interesting to see if improvement occured with higher dosages. Initial improvement may be slow, with benefit in some cases taking a number of months to occur. A trial period of 6 months therapy should, therefore, be tried before abandoning therapy. Diabetic patients should be forewarned to monitor their control closely and modify their insulin accordingly.

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Nail dystrophy in chronic sarcoidosis

N.H.COX AND D.J.GAWKRODGER*

Department of Dermatology, Royal Victoria Infirmary, Newcastle, NE1 4LP and *Department of Dermatology, Royal Infirmary, Edinburgh EH3 9YW, U.K.

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SUMMARY

We report details of five patients with nail dystrophy associated with sarcoidosis. All had bone cysts in the underlying terminal phalanx and had a chronic course of the disease. Radiological examination is indicated in patients with sarcoidosis and nail dystrophy as bone cysts are frequently asymptomatic.

Nail dystrophy in sarcoidosis is either rare or ignored, and only a small number of cases has been reported. Although nail abnormalities may be associated with lupus pernio of the adjacent digit, or with sarcoidal dactylitis, they may also occur without clinically apparent involvement of adjacent soft tissue. However, digital bone cysts have been found in almost all previously reported cases of sarcoidal nail dystrophy. Although the overall frequency of bone cysts in sarcoidosis is low, and they are frequently asymptomatic, their presence implies a chronic course of the disease. We report five cases of sarcoidosis with nail dystrophy and discuss the implications of this diagnosis.

CASE REPORTS

Case 1

A 64-year-old Caucasian female had longitudinal ridging, thickening and fragility of nails of the thumbs and some fingers. She had a 20-year history of sarcoidosis characterized by lupus pernio of the face (associated with nasal cartilage destruction) and of the hands, with fusiform swelling of the right ring finger. For 12 months she had suffered exertional dyspnoea and had developed radiological evidence of pulmonary fibrosis, and decreased transfer factor. Serum angiotensin converting enzyme (SACE) was elevated (126 U/l, normal 0–88 U/l). Cysts were demonstrated radiologically in the terminal phalanges of the digits affected by nail dystrophy (Fig. 1) and in the proximal phalanx of the right ring finger.

Correspondence: Dr Neil H. Cox.

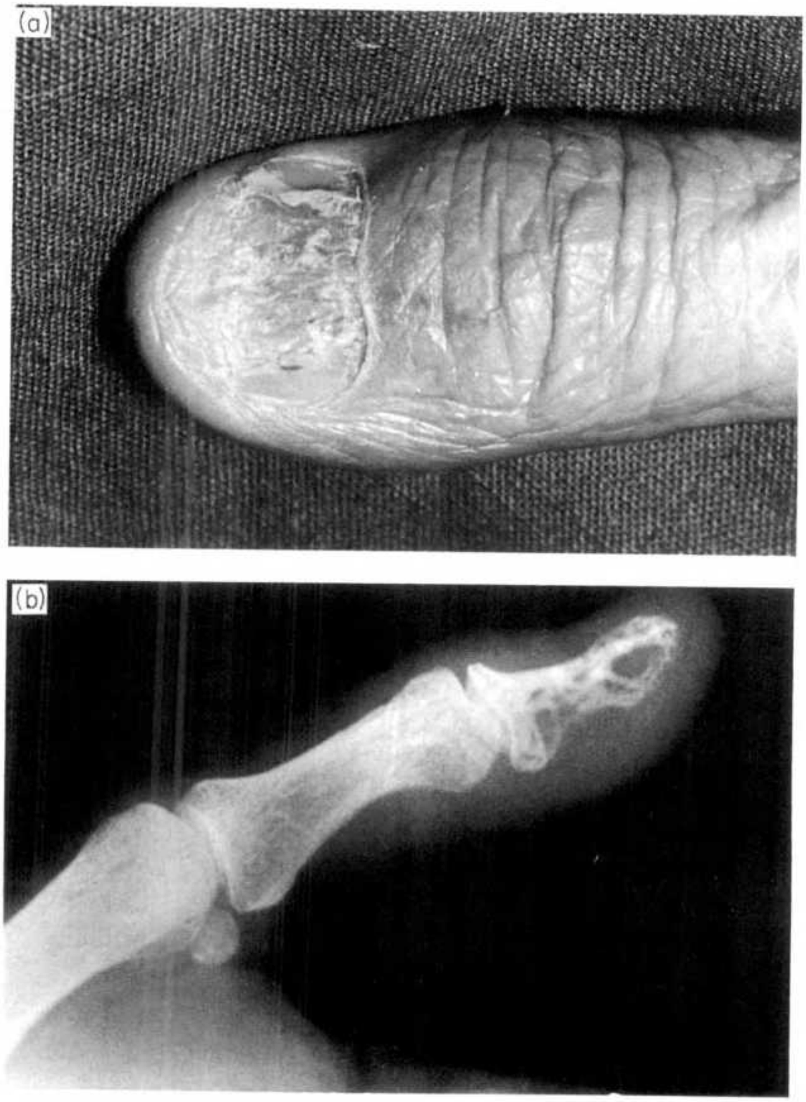

FIGURE 1. Thumb of Patient 1. (a) Clinical appearance. (b) Radiograph.

Case 2

A 46-year-old Caucasian male was referred for dermatological advice regarding dystrophic changes in five nails. Two years previously he had developed dystrophy of the left hallux nail and swelling of the left second toe. At that time, the bone of the terminal phalanx of the second toe was curetted and histological examination demonstrated numerous granulomata with no acid fast bacilli. A diagnosis of tuberculosis had been considered, but healing occurred without specific therapy. When referred 2 years later he had lupus pernio affecting the left second toe, and swelling of the distal left middle finger. The nail of this finger had a longitudinal ridge with atrophy of the ulnar aspect, pink discolouration, fragility and onycholysis. Three other fingernails were dystrophic and had splinter haemorrhages or irregular pink or brown macular discolouration. Radiological examination demonstrated a lace-like trabecular pattern of the digital bones, well defined lucent areas in the terminal phalanges of five fingers and three toes, and a pathological fracture involving the terminal phalanx of the left middle finger (Fig. 2). A tine test was negative. Review of the bone histology revealed changes entirely consistent with a diagnosis of sarcoidosis.

Case 3

A 64-year-old Caucasian female developed uveitis, hilar lymphadenopathy, splenomegaly and probable erythema nodosum in 1969. Five years later she was noted to have longitudinal cracking and ridging of both thumb nails. Radiographs of the hands demonstrated evidence of altered bony texture compatible with sarcoidosis, and early cyst formation was observed in the

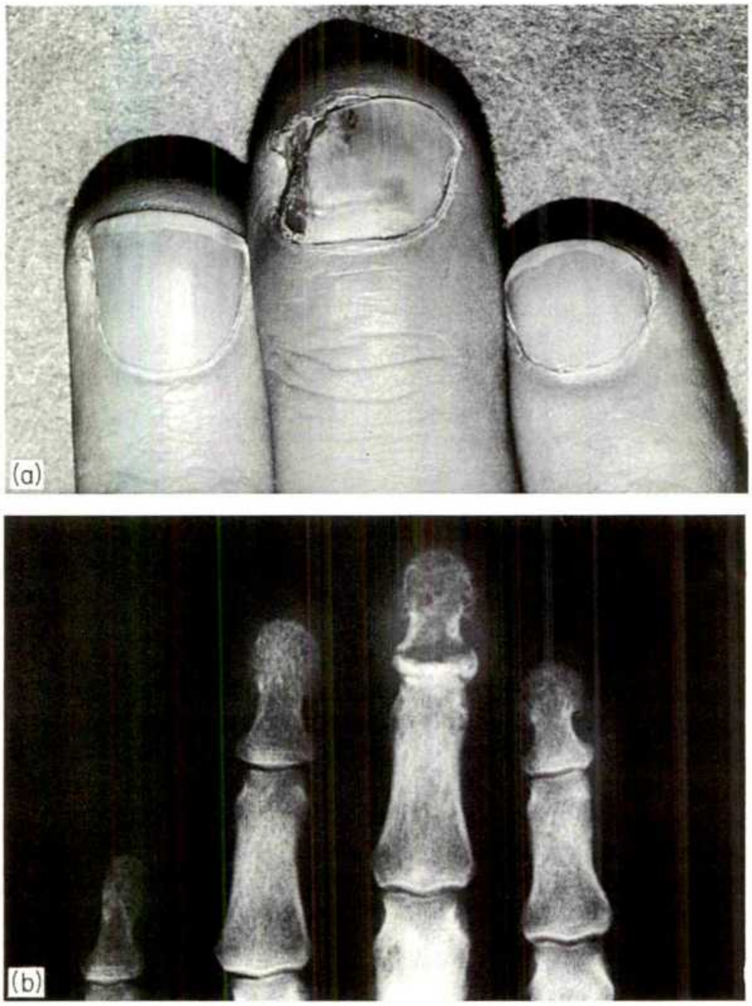

FIGURE 2. Left middle finger of Patient 2. (a) Clinical appearance. (b) Radiograph.

terminal phalanx of the thumb with the more severe nail dystrophy. Secondary infection and formation of granulation tissue at the proximal nailfold caused considerable morbidity. Subsequently she developed an ulcerated plaque on the lower leg, thought to be lupus pernio, and suffered gradually increasing impairment of respiratory function. Treatment of her respiratory symptoms with oral prednisolone resulted in improvement in her nail dystrophy.

Case 4

A 44-year-old Fijian man developed longitudinal ridging and cracking of several toenails. He had a 10-year history of sarcoidosis, diagnosed initially when he was found to have asymptomatic hilar lymphadenopathy. He subsequently developed fusiform swelling of the phalanges of the hands and feet, and lupus pernio of the right second toe. Routine blood biochemistry and SACE were normal. Radiographs showed cystic changes and an altered trabecular pattern in several phalanges.

Case 5

A 42-year-old Caucasian male presented with lethargy, cold intolerance, dry skin, decreased beard and body hair growth, impotence, polydipsia and polyuria. Nasal sarcoidosis had been diagnosed 8 years previously and confirmed histologically. At presentation he had lupus pernio of the nose, nasal cartilage destruction, and a few chronic sarcoidal plaque lesions of the skin. A Kveim test was positive and a Mantoux test at 1:1000 was negative. Extensive endocrinological investigation demonstrated abnormalities consistent with the diagnosis of hypothalamic sarcoidosis. He also had a 12-year history of splitting and onycholysis of the right index fingernail, and similar changes of the left hallux nail for 3 years. The nailfolds of these two digits were painful, erythematous and swollen. Milder changes were apparent in the nails of both little fingers. Radiological examination demonstrated coarse trabeculation and cyst formation in the terminal phalanges of the right index and left ring fingers. Over a period of 5 years his nail dystrophy progressed until there was almost complete destruction of the right index fingernail with pterygium formation, and severe longitudinal ridging and splitting of grossly hyperkeratotic hallux nails bilaterally.

DISCUSSION

The dystrophic changes of the nail plate in sarcoidosis include thickening, opacity, fragility, layering, convexity, longitudinal ridging, pitting, atrophy and nail loss.[1–6] Red or brown discolouration of the nail beds[2] and splinter haemorrhages also occur.[1] One patient with biopsy-proven sarcoidosis of the nail matrix developed pterygium,[7] in which proximal nailfold skin becomes adherent to the nail bed. Finger clubbing usually indicates secondary bacterial infection in patients with long-standing pulmonary fibrosis,[6] but sarcoidal infiltration of the nailfolds and adjacent skin may cause swelling and fissuring,[2] and can be associated with lupus pernio of the fingers. The changes sometimes improve after therapy with prednisolone and chloroquine,[3] but improved after treatment with oral prednisolone alone in one of our patients.

Nail dystrophy occurred in only one of 400 patients with sarcoidosis studied by Patel and Sharma,[3] and in three of 188 patients with cutaneous sarcoidosis studied by Veien *et al.*[8] However, it is more frequent in patients with sarcoidal dactylitis, occurring in one of four patients reported by Pitt *et al.*[9] and three of six described by Leibowitz *et al.*[10] A particularly mutilating form of sarcoidosis has been illustrated by Morrison,[11] in which there is gross bony destruction, extensive skin lesions and prominent nail dystrophy. Overall, nail dystrophy is rare in sarcoidosis, but is important because it is associated with bone involvement and other features of chronic sarcoidosis. In the patients we have described, there is a clear relationship between nail dystrophy and bony involvement in the underlying terminal phalanx of the individual digits affected. All reported patients with sarcoidal nail dystrophy have had bony changes, with the exception of one patient described by Liebowitz *et al.*[10] This patient had facial lupus pernio, lung fibrosis, and ulceration and soft tissue swelling of the fingers associated with nail dystrophy, but no radiological changes. Phalanges of the hands and feet are frequently involved in patients who have sarcoidosis of bone, although almost half of the 29 patients studied by Neville *et al.*[12] were asymptomatic. Two-thirds of these patients had chronic skin lesions, including lupus pernio in 48%, and 86% had evidence of pulmonary disease. Bone cysts were present in 43% of patients with lupus pernio studied by Spiteri *et al.*,[13] but were found in only 4% of all their patients with sarcoidosis.

The patients described in the present report emphasize the clear relationship between nail dystrophy and bony involvement of the underlying terminal phalanx. Other disorders in which nail dystrophy is associated with local bony abnormalities include tumours such as exostoses, enchondromas, glomus tumours and keratoacanthomas, as well as systemic diseases such as tophaceous gout, thyrotoxicosis, systemic sclerosis and vinyl chloride disease. The nail dystrophy of sarcoidosis is probably more common than the few reported cases would suggest, and it is a useful indicator of disease chronicity. As bony changes are asymptomatic in about 50% of affected patients, but are of prognostic importance, the presence of nail dystrophy in patients with sarcoidosis is an indication for radiological examination of the hands and feet.

ACKNOWLEDGMENTS

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Delayed wound healing and keloid formation following argon laser treatment or dermabrasion during isotretinoin treatment

H.ZACHARIAE

Department of Dermatology, University of Aarhus, DK-8000 Aarhus C, Denmark

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SUMMARY

We report the observation of delayed wound healing and keloid formation in three patients, following dermabrasion or Argon laser treatment administered while they were receiving isotretinoin for acne or rosacea.

The introduction of isotretinoin (13-cis-retinoic acid) for the treatment of severe recalcitrant acne has dramatically altered the prognosis of this often troublesome and disfiguring disease.[1] More recently isotretinoin has also been used for rosacea.[2] Not only does isotretinoin produce marked clearing; even more impressive are the prolonged remissions which have been observed. A number of patients with severe acne however, have acne scars, for which dermabrasion may be used.[3] In rosacea, telangiectasias may be treated with an Argon laser.[4] In both cases healing usually takes place rapidly and without keloid formation, unless the patient is prone to produce keloids. This can often be established by a personal or family history. We report here delayed wound healing and keloid formation in three patients treated with dermabrasion or with an Argon laser during isotretinoin administration for acne or rosacea.

CASE REPORTS

Case 1

A 25-year-old female with long-standing facial telangiectasis of the rosacea type was referred to our department in November 1985. She was treated with Argon laser 1·3 W 0·2 s pulse therapy, first administered to a 1 × 1 cm test area on the left cheek using a Lexel Aurora model 150 (Optilas, Rijn, Holland), in December 1985. Neither this test treatment nor subsequent treatment of the right cheek in January 1986 gave rise to scarring. Some minor erosions healed within a week. Figure 1 shows the patient's left cheek after this first laser treatment.

In March 1986 she received isotretinoin (Roaccutan®) 60 mg daily for her rosacea and in April 1986 she underwent further 1·3 W 0·2 s pulse laser therapy on the most severely affected areas.

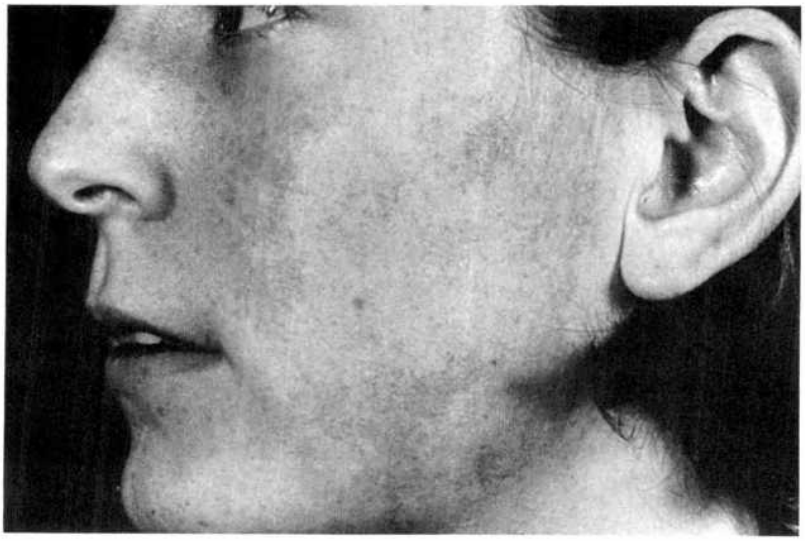

FIGURE 1. Case 1. Appearance of the left check after the first treatment. Note the complete absence of scarring

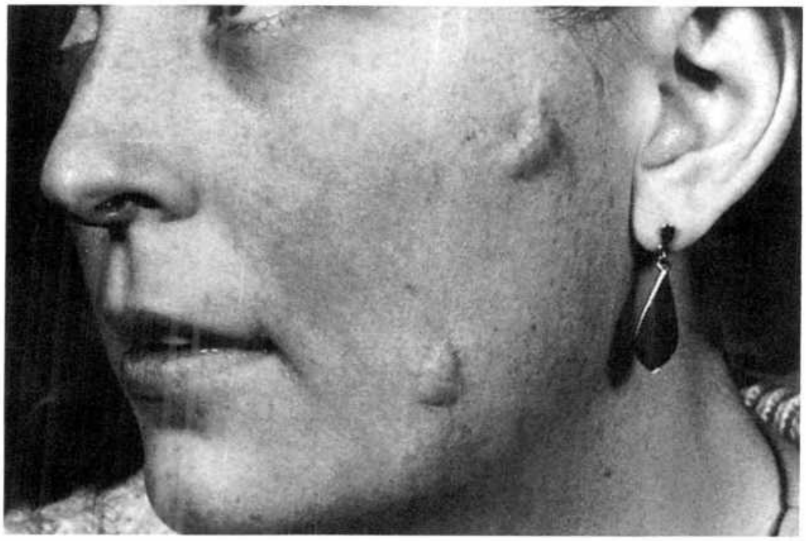

FIGURE 2. Case 1. Appearance of the left cheek following argon laser treatment during isotretinoin therapy. Note the two keloids.

This time, however, the lesions took more than 8 weeks to heal and after approximately a further 4 weeks the scars became raised and thickened and 4 weeks later well-defined keloids were present (Fig. 2). We suspected that the isotretinoin treatment could be partly responsible for the delayed wound healing and so Roaccutan was discontinued in May 1986. On a thorough examination of the patient's skin, we found a small keloid on her left knee indicating a tendency in this patient to form keloids.

Case 2

A 27-year-old female acne patient was referred to our department in September 1985 for dermabrasion of a large traumatically induced scar on her left cheek. She displayed no signs of keloid formation and there was no family history of keloids. Dermabrasion was performed according to our standard procedure[3] under local freon anaesthesia. Healing took place without complications within 2 weeks.

In September 1986 she was readmitted for further dermabrasion of the scar. At this time she was receiving treatment with Roaccutan® 60 mg daily for her acne. This medication was not discontinued and dermabrasion was performed, according to the same procedure as before, in September 1986. After this second dermabrasion, however, complete healing did not take place until mid-December 1986. This is in contrast to a normal healing time of approximately 10 days after this type of operation. We saw the patient again 6 months later, when she was referred to us by her local dermatologist with a keloid in the treated area.

Case 3

A 70-year-old male suffering from rosacea and rhinophyma was referred to us for dermabrasion of the nose, which was performed in September 1986. Immediately after dermabrasion he was given isotretinoin 60 mg daily for his rosacea. Healing took 4 weeks, and at routine follow-up 8 weeks after the operation, keloid formation was diagnosed on the right side of the nose. Isotretinoin was, therefore, discontinued and his previous rosacea treatment with tetracycline reintroduced. This patient had no signs of previous keloids and he had earlier been given five Argon laser treatments for telangiectasis, without scarification.

DISCUSSION

The present findings are similar to those of Rubenstein *et al.*[5] who found hypertrophic scarring or keloids in six patients who underwent dermabrasion while on or having recently completed isotretinoin therapy. We feel that our cases provide particularly convincing evidence as each patient to a certain extent acted as his or her own control.

Retinoids have been shown to modulate connective tissue metabolism in keloid fibroblast cultures,[6] and suppression of production of collagenase by retinoids may be responsible for the keloid formation observed in our patients. The problem is, however, complex. Isotretinoin selectively inhibits normal skin fibroblasts[7] and under certain conditions decreases collagen formation *in vitro*.[8] Topical retinoids have therefore been suggested for treatment of keloids.[9]

Another important factor could be delayed epithelialization. In most *in vitro* studies epidermal cell growth has been found to be reduced[10] and delayed wound healing may subsequently lead to scar hypertrophy. Post-operative wound healing has been reported to be adversely affected by retinoids. Taking these factors into consideration together with the present cases and those previously reported[5] it is probably wise to suggest, that, when possible, surgical procedures in retinoid treated patients be postponed until after discontinuation of therapy and until the activity of the retinoids has had time to subside.

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Contact urticaria to locusts

B.E.MONK

Department of Dermatology, Bedford General Hospital, Kempston Road, Bedford MK42 9DJ, U.K.

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SUMMARY

We describe a case of allergic contact urticaria provoked by locusts in a laboratory research worker. One of her colleagues also had locust-induced contact urticaria, and another had asthma provoked by locust exposure. Contact urticaria to locusts is an occupational hazard apparently well known to professional entomologists, but one which has not previously been described in the dermatological literature, to my knowledge.

CASE REPORT

The patient was a 23-year-old research biochemist working in a university zoology department on the mechanism of action of neurotoxins in the locust. Her work regularly required her to enter the insect house in which a large breeding colony of locusts was maintained. She had a history of mild hayfever and asthma in childhood, but these had been in remission for some years. Within a few weeks of starting work in the department she had noted that whenever she entered the locust house, exposed areas of skin would begin to itch, swell, and go red, the changes occurring within a few minutes. Direct physical contact with the locusts would produce local swelling within 2 min. The symptoms abated within a few hours, and disappeared totally during the university vacation when she was away from work. Protective clothing gave some relief. Four other students worked in the same department; two were symptom free, but two gave histories of an immediate-type of reaction on entering the locust house. In one subject this was manifested as asthma, and in the other as conjunctivitis and contact urticaria. In the latter instance the symptoms were of a severity sufficient to cause the subject to abandon her research project; this was followed by a resolution of the symptoms.

The patient was investigated by prick testing, and this showed positive reactions to grass pollens, house dust mite, cockroach antigen (Dome Hollister-Stier), and locust antigen, (prepared by the Brompton Hospital, London, according to the method of Burge *et al.*[1]). Her total blood IgE level was mildly elevated at 302 IU/l. A direct provocation test with a live locust produced an immediate wealing reaction at the sites of contact, developing within 1 min. No reaction was seen in a control subject with no previous history of locust exposure.

DISCUSSION

Locusts are swarming migratory grasshoppers whose destructive effects on crops and vegetation have been known since biblical times, and continue to have a devastating impact on many of the poorest countries on earth. Sensitivity reactions including contact urticaria provoked by locusts and their debris are well recognized as an occupational hazard by professional entomologists,[2] and may be severe enough to cause the subject to abandon working with locusts. The problem is apparently rarely encountered in the field, even when locusts are in dense swarms, but is common in research centres where large numbers of locusts are maintained in breeding colonies, usually in relatively confined spaces. The problem of locust sensitivity was first investigated by Frankland[3] who studied workers in two research centres, and found that a number gave a history of asthma, rhinitis and in three cases contact urticaria. Burge *et al.*[1] studied workers in one of the research centres which had been included in Frankland's original study and found a high incidence of work related asthma, rhinitis, and contact urticaria, the latter symptom being present in one third of subjects. Positive prick tests to locust antigen were present in affected subjects; marked cross reactivity was also shown to cockroach, an entomologically related species which itself may provoke hypersensitivity reactions.[4] Atopic subjects were more commonly, but not exclusively, affected. The allergen has now been identified as the peritrophic membrane of the midgut, which surrounds the faeces and is dispersed through the air in an enclosed environment; specific IgE to this allergen has been demonstrated in the blood of affected individuals.[5]

The propensity of locusts to provoke sensitivity reactions is sufficiently well known among professional entomologists that it is unlikely to cause diagnostic difficulty. Locusts are, however, also widely used in the teaching of biology in schools and colleges where their potential for inducing cutaneous reactions may not be appreciated.

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Kaposi's sarcoma in kidney transplant recipients treated with cyclosporin

P.L.BENCINI, L.MARCHESI,* T.CAINELLI* AND C.CROSTI

Università degli Studi di Milano, Clinica Dermatologica Ospedale San Paolo, and *Clinical Dermatologica Ospedale Maggiore di Bergamo, Milan, Italy

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SUMMARY

We report the clinical and histological features and the course of Kaposi's sarcoma in three transplant patients treated with cyclosporin. Four months after cyclosporin treatment was stopped the lesions in all three patients had completely healed.

At present at least four different types of Kaposi's sarcoma (KS) are well recognized. These are sporadic Kaposi's sarcoma, endemic KS in African countries, epidemic KS related to AIDS and KS in patients on immunosuppressive therapy.[1]

In the last 10 years there have been several reports of KS in patients with various disorders treated with immunosuppressive agents,[2,3] the most frequent being after renal transplantation.[4] KS has been reported in kidney transplant recipients treated with azathioprine plus a steroid.[5] We report here the clinical and histological features, the course and the treatment of KS in three kidney transplant patients treated with cyclosporin. The patients were seen during long term surveillance for skin cancer of a large series of kidney transplant patients treated with cyclosporin.

CASE REPORTS

Immunosuppressive regimen

The patients were treated with cyclosporin plus a steroid. The immunosuppressive regimen was i.v. cyclosporin 5 mg/kg/day on 4 consecutive days, starting the day before surgery, followed by oral administration of 15 mg/kg/day, decreased every 2 weeks by 2 mg/kg/day to a maintenance dose of 7 mg/kg/day, in combination with 120 mg/day methylprednisolone i.v., for 4 days followed by 16 mg/day, orally, tapered off to 8 mg/day over 6 months.

One patient (Case 3) was treated also with azathioprine, at a maintenance dose of 50 mg/day.

Correspondence: Pier Luca Bencini, Clinica Dermatologica Ospedale San Paolo, Via di Rudini 8, 20142 Milano, Italia.

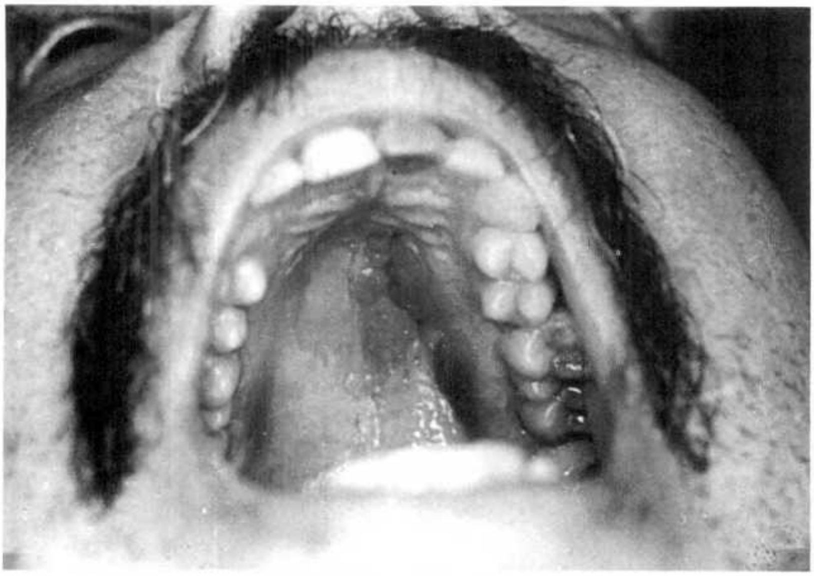

FIGURE 1. Case 1. Violaceous plaque on the hard palate.

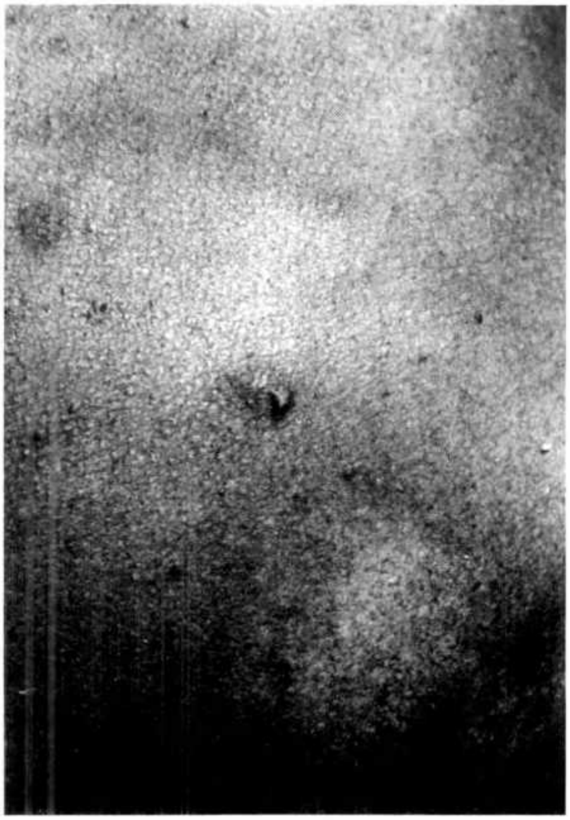

FIGURE 2. Case 2. Isolated angiomatous lesions on the right hip.

Case 1. This 39-year-old man was placed on periodic haemodialysis at the age of 33 for chronic renal insufficiency due to renal hypoplasia. At the age of 38 he was given a cadaver renal transplant. Five months later he developed two basal cell carcinomata on his head which were treated surgically. At the end of the first year after transplantation a dermatological examination revealed several, small, isolated, purplish, nodular and flat lesions, some of them resembling bruises, scattered over the trunk, abdomen, arms and head. There were also two violaceous plaques on his right arm and on the hard palate (Fig. 1). Two biopsies were taken, one from a flat lesion and one from a nodular lesion. Histology confirmed the diagnosis of KS. An examination revealed that the pharynx was also involved by KS. Further clinical, laboratory and radiographic examinations did not reveal any other visceral involvement.

Case 2. This patient, a 40-year-old man, developed chronic renal insufficiency at the age of 36, due to diabetic nephropathy. After 3 years of haemodialysis, he was given a cadaver renal transplant. Ten months later a dermatological examination revealed a few isolated, red-brown, flat lesions, slightly infiltrated, on the hips, thighs and thorax (Fig. 2). Two small lesions were also present in the mouth. Histology confirmed the diagnosis of KS. Clinical, laboratory and radiographic investigation did not reveal any visceral involvement.

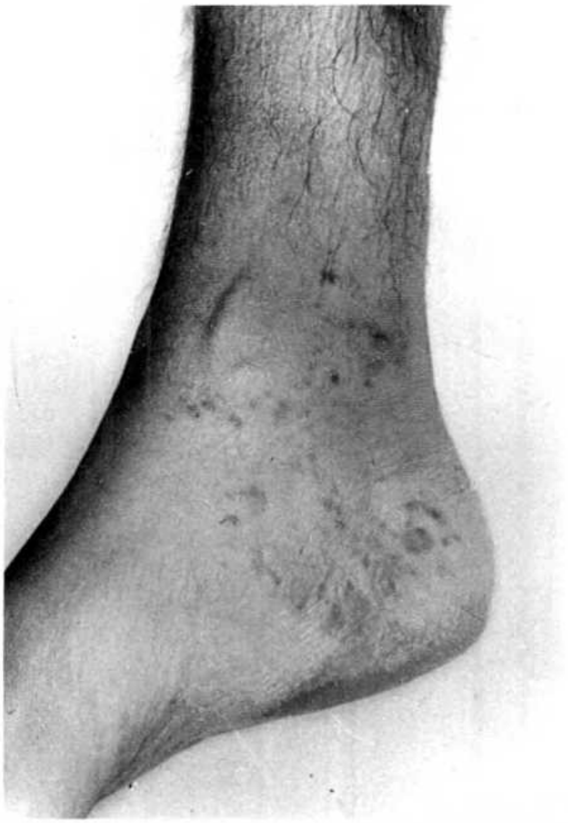

FIGURE 3. Case 3. Several papulo-nodular and flat spots on the right leg and foot.

Case 3. This 35-year-old man was given cadaver kidney transplant at the age of 31 because of chronic renal failure due to chronic glomerulonephritis. Four years after transplantation, a dermatological examination was carried out because of the appearance 3 months earlier of some angiomatous spots on his right leg and foot. The lesions were multiple, firm, smooth, purple, non-tender, papulonodular and flat (Fig. 3). The examination also revealed two flat, smooth, red, roundish lesions, approximately 0·9 cm in diameter, in the oral mucosa. The pathology report on one leg lesion and one oral lesion confirmed the diagnosis of KS. Clinical, laboratory and radiographic investigations did not reveal any visceral involvement.

Clinical course

All three patients were taken off the immunosuppressive drugs, remaining only on 8 mg/day methylprednisolone. In all the patients, we observed a progressive flattening of the mucocutaneous lesions, and the lesions were completely healed by 4 months after the suspension of therapy. Renal function was conserved in all patients.

Histology

Florid lesions. In all the specimens there were increased numbers of dilated blood vessels in the dermis and a proliferation of spindle-cells that extended irregularly up to the lower part of the dermis. There were several narrow slits containing many erythrocytes among the spindle-cells. Deposits of haemosiderin were also evident within the lesions. There was a lack of the diffuse chronic inflammatory infiltrate that is usually found in the classical form of KS (Fig. 4).

Resolving lesions. A resolving lesion in one patient was biopsied. We observed a gradual reduction of the spread of both the vascular and the spindle-cell proliferations, in association with a progressive re-appearance of the inflammatory reaction, consisting of scattered lymphoid cells, plasma cells and a few histiocytes (Fig. 5).

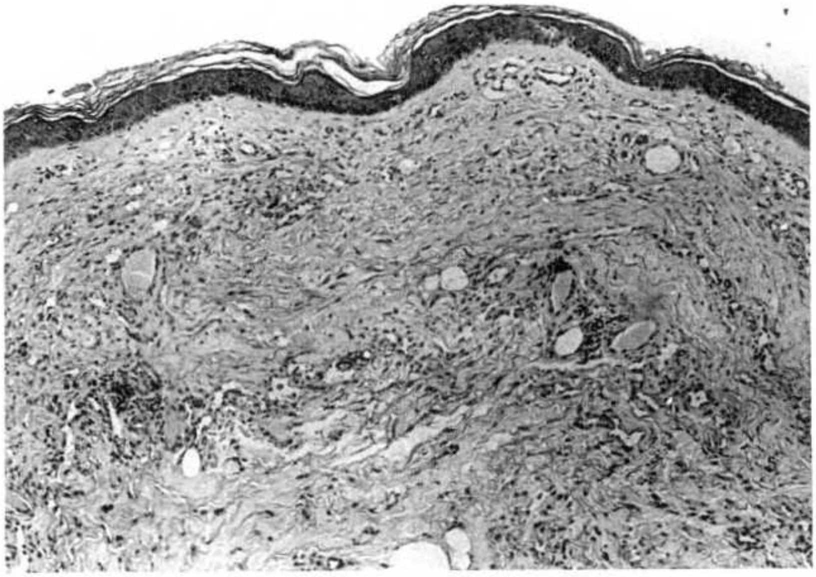

FIGURE 4. Case 1. Histology of a florid lesion.

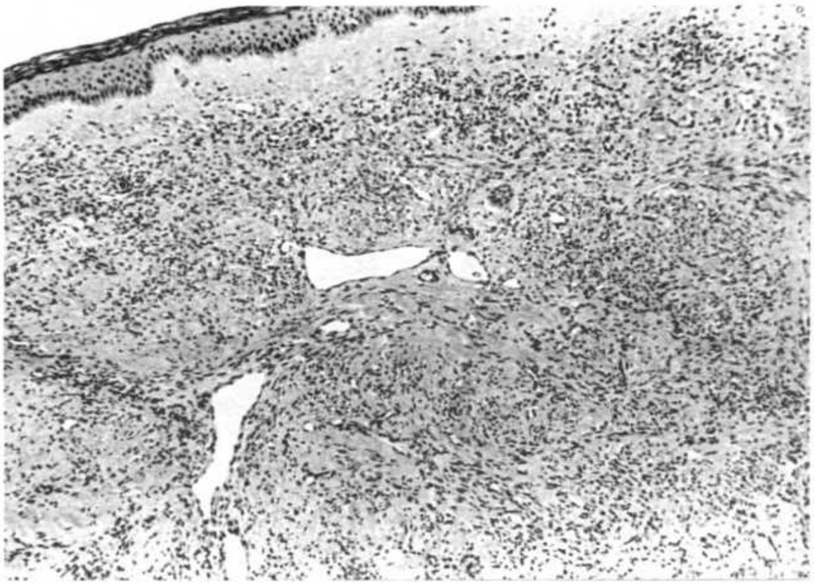

FIGURE 5. Case 1. Histology of a resolving lesion 30 days after suspension of the immunosuppressive therapy.

DISCUSSION

Kidney transplant recipients have been reported to have a high incidence of several neoplasias; KS represents approximately 3% of these. Moreover, some investigators have found 400 to 500 times as many cases of KS in transplant patients as in the general population.[5,6] The immunological background of these patients makes this kind of KS different from the classical type. The high incidence, the eruptive course of the lesions, and the frequent occurrence of visceral involvement indicate a relationship between this neoplasia and the epidemic KS observed in AIDS patients. In 1983 we described only one case of KS in a report on a large series of kidney transplant recipients treated with azathioprine and steroids.[7]

It appears that this relationship is more evident in patients treated with cyclosporin, both in the clinical aspects of the disease and in the higher incidence of KS in this group than in conventionally treated patients. This hypothesis is supported by an ultrastructural study which showed the presence of tuboreticular cytoplasmic structures only in cyclosporin-treated patients, and not in conventionally treated patients.[9] These structures are similar to those observed in the AIDS-related form of the disease.[10]

Histology of lesions in our patients showed that there was a sparse chronic inflammatory infiltrate in the florid lesions while there was a progressive reappearance of the inflammatory reaction after immunosuppressive therapy was stopped. To our knowledge, there are no other reports in the literature describing these histological features. If confirmed by other studies, these features may provide important supporting evidence for the primary role of immunosuppression in the pathogenesis of this form of KS. Many types of therapy have been advocated and tried in KS.[11] Irradiation, surgery and chemotherapy have been used, with varying degrees of success, but often after single treatments there are recurrences and progression of the disease.[12-14] The response of the KS in these patients to radiotherapy is less predictable, this treatment producing lower response rates than it does in patients without a transplant.[5,15] As the combination of several immunosuppressive drugs is important in the pathogenesis of KS in these patients, we think, as do other observers,[16-18] that the reduction or suspension of the immunosuppressive therapy is the treatment of choice for this tumour.

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Aplasia cutis congenita presenting as a familial triad of atrophic alopecia, ocular defects and a peculiar scarring tendency of the skin

R.S.C.LEUNG, W.E.BEER AND H.K.MEHTA

Departments of Dermatology and Ophthalmology, Ysbyty Gwynedd, Bangor, North Wales, U.K.

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SUMMARY

We report two siblings with atrophic alopecia, ocular defects and an abnormal tendency to cutaneous scarring. To our knowledge this appears to be the first report of this familial oculocutaneous association and of this peculiar cutaneous scarring.

CASE REPORTS

Case 1

A 18-year-old Caucasian female, attended the Dermatology Clinic in 1975. She was the eldest of three children. Her mother's pregnancy had been complicated from the 6th month by recurrent antepartum haemorrhages. Her mother had received no specific treatment. Vaginal vertex delivery at 41 weeks was normal with no neonatal problems.

At birth her mother noted a 'blood blister' on the vertex which later disappeared leaving a small bald area. The parents were concerned about her eyesight from early childhood; they noted that she frequently tripped over objects, held books near to her face and sat very close to the television set.

A tendency to develop permanent scars after minor injuries was noted from the age of 10 months. The actual healing process appeared to be normal and there was no blistering.

Growth, psychomotor and sexual development were normal and she performed well at an ordinary school. Her parents came from the same area of Anglesey, North Wales, but were unrelated. A paternal first cousin, once removed, was a tyrosinase positive oculocutaneous albino. A maternal cousin, allegedly, had poor eyesight in one eye. We were, however, unable to examine this relative.

On examination, the patient had a patch of cicatricial alopecia over the vertex measuring 15 cm in diameter (Fig. 1). There were multiple atrophic, macular, linear and hypopigmented scars

Correspondence: Dr R.S.C.Leung, The Institute of Dermatology, 5 Lisle Street, London WC2H 7BJ, U.K.

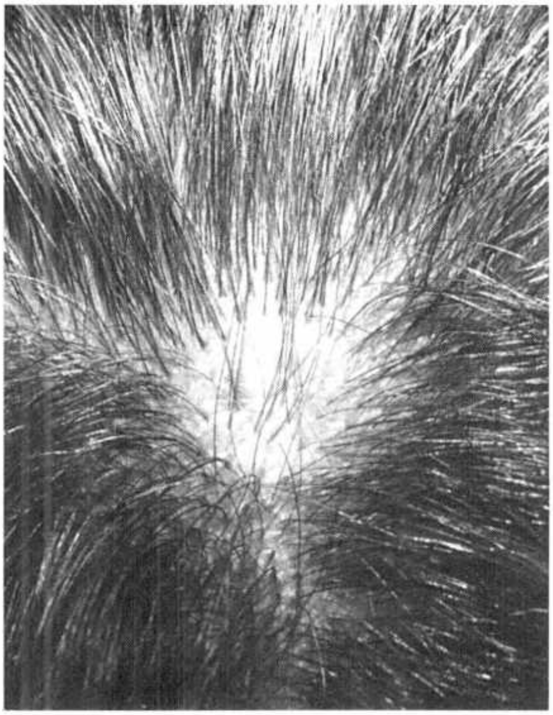

FIGURE 1. Case 1. Circumscribed atrophic alopecia on the vertex.

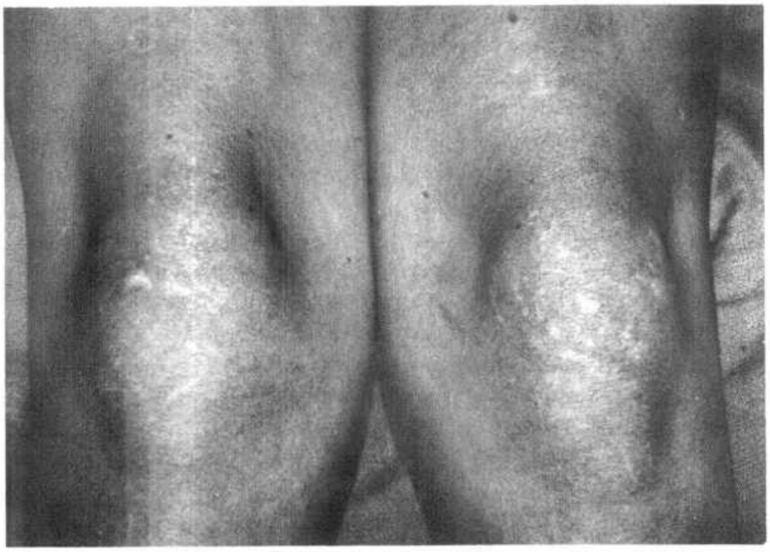

FIGURE 2. Case 1. Macular and linear scars on the knees.

over the knees (Fig. 2), knuckles and to a lesser extent over the elbows and the trunk. There was a keloid over the vaccination scar on the left upper arm. The oral mucosa, hair and nails were normal. There was no evidence of hyperextensibility of the skin or joints. Mild dermographism was demonstrated and plane warts were observed on the dorsa of the hands. A milium on the right elbow and several possibly growth-related striae on the lower trunk had appeared when she was aged 8 and 14, respectively.

Ophthalmic examination revealed bilateral myopia, keratoconus, atrophic irides, lamellar cataracts and vitreous detachment. The left eye showed evidence of posterior synechia—without active uveitis—and retinal detachment. The visual acuity with myopic correction was 6/36 in the right eye and hand movements in the left eye, which lost vision completely over the following 8 years. At the time of the present report she had no perception of light in the left eye, and had also developed a full cataract.

The following investigations were negative or normal: Full blood count, ESR, VDRL, TPHA, serum copper, C_3, C_4, total haemolytic complement, urinary galactose and urinary amino acid screen. Blood, stool and urine screens for porphyrins were negative. A scar was biopsied and the histology showed possible mild thickening of dermal collagen; no other distinctive abnormality was seen. Von Kossa, PAS stain for elastic tissue and electron microscopy were unhelpful. The collagenase and hydroxyproline content of a tissue culture from the biopsy were normal in comparison with age matched control fibroblasts. Fibroblast culture, collagen pattern and chromosomal studies were all normal. Suction blisters on the abdomen occurred at 83 min on the left side and at 93 min on the right; both results were within the normal range.

Case 2

Case 2 was the 8-year-old brother of Case 1. This third pregnancy had been uncomplicated. Labour was induced at 10 days post-term resulting in a forceps delivery. He was born with a red patch over the vertex, which later disappeared leaving a bald patch. He developed visual problems like his sister, with the result that he was frequently tripping over objects. A scarring tendency after trivial injuries became apparent when he started walking. He underwent a routine circumcision at the age of 1 year. This was complicated by a symptomatic phimosis from a constricting band, proximal to the preputial free edge. A second successful circumcision was performed 2 years later. Biopsy of the phimotic prepuce showed fibrosis of an exuberant type. He suffered from mild asthma and eczema. Psychomotor, sexual and intellectual development were entirely normal.

On examination, a patch of atrophic alopecia was noted over the vertex (Fig. 3). His scalp and hair were otherwise normal. He had linear, atrophic and macular scars on his hands, forearms and lower legs, similar to those of his sister. In addition, he had eczema in both antecubital and popliteal fossae and on the upper medial thighs. A wart was noted on the right hand. Ophthalmic examination at the age of 20 months revealed him to be a high myope with fluid vitreous and atrophic pigment epithelium. He had central ocular nystagmus. He did not have cataracts or evidence of uveitis or retinal detachment. His vision remained poor at 2/60 in each eye, and he has been registered 'blind'. Similar investigations to those carried out in Case 1 were performed and were all normal.

The 15-year-old sister, of Cases 1 and 2 had no ocular problems, but had a few very small keloidal scars on the knuckles and the dorsa of the hands. Otherwise no abnormality was apparent.

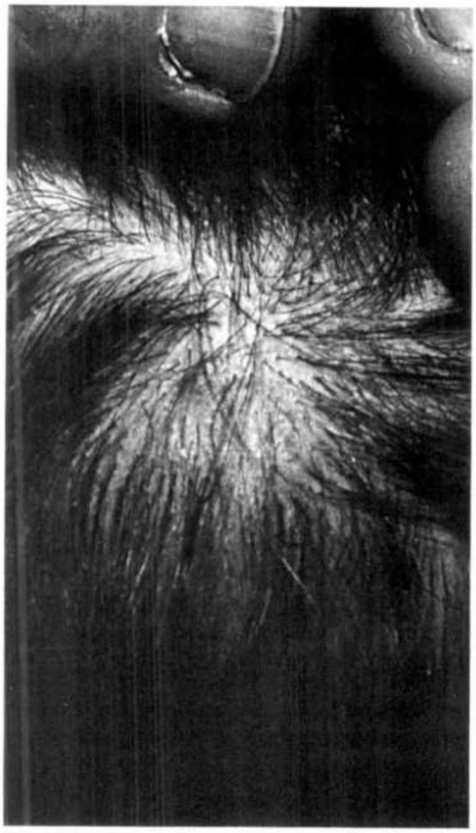

FIGURE 3. Case 2. Circumscribed atrophic alopecia on the vertex.

DISCUSSION

Aplasia cutis congenita (ACC) refers to congenital absence of epidermis and, on occasion, hypodermis from one or more areas.[1] The diagnosis is primarily clinical with a variable histological appearance.[2]

The scalp is the commonest site involved, accounting for over 80% of solitary lesions.[3] The lesions are most commonly circular, but may be oval, linear or rhomboidal or even have stellate configurations. They are commonly present at birth as superficial erosions or deep ulcerations and sometimes the lesions may be covered by a membranous epithelium filled with serous fluid resembling a blister, as in Case 1. Healing with scar formation follows within weeks or months and rarely results in keloidal healing.[4]

The aetiology of the condition is unknown and a unifying theory is lacking, probably reflecting the heterogeneity of the disease. Tearing off of foetal skin, secondary to amniotic adherence, intra-uterine trauma and infection have apparently been responsible in some cases.[3,5] It has also been reported in infants whose mothers took methimazole, a drug used in the management of thyrotoxicosis.[6] The fact that vertex is the point of maximum tensile forces during rapid brain growth in early foetal organogenesis is thought to be responsible for the predilection of ACC to be near the hair whorl on the vertex.[7]

A large number of associated abnormalities have been described with ACC of the scalp; among these are cleft lip and palate, tracheo-oesophageal fistula, patent ductus arteriosus, polycystic kidney, mental retardation and limb reduction abnormalities. Ocular abnormalities including corneal opacities, scleral dermoids and eyelid colobomas were found in a group which had epidermal and organoid naevi.[2] Other authors also described ocular colobomata and white rings on the cornea in association with the condition.[8,9]

A good review of ACC of the scalp was published recently by Frieden.[2] He proposed a clinical classification of the syndrome into nine groups. It seems to us that ACC of the scalp should perhaps be treated as a specific entity, and thus distinguished from aplasia of known causes such as epidermolysis bullosa and focal dermal hypoplasia, which may, in addition to the cutaneous defects on the trunk and limbs, also affect the scalp. We note that a similar observation was made by Ingalls.[10]

The scarring tendency of our two cases is very unusual, and does not seem to fall into any known form of atrophic dermatosis. The lesions in our cases were clearly related to minor trauma and there was no preceding relapsing erythematous phase or infection as in post-inflammatory elastolysis[11] or secondary macular atrophy.[12] Regarding the scalp atrophy, we must add that no pH electrodes were applied at birth.

Cataracts in childhood is a rare condition and has many causes of which the commonest is idiopathic.[13] Many dermatoses including atopic eczema and anetoderma have been reported to be associated with cataracts.[14–16] Myopia is relatively common and we do not claim that this is a definite part of ACC unless it can be shown that other reported cases of ACC have a higher incidence of myopia than normal.

Both autosomal dominant and recessive modes of inheritance have been reported with ACC. The occurrence of this oculo-cutaneous abnormality in two siblings and the involvement of both sexes would suggest an autosomal recessive mode of inheritance in these cases.

This familial triad of ACC of the scalp, ocular defects and the curious tendency to scar on the trunk and limbs in response to minor trauma, has not been described previously, to our knowledge.

ACKNOWLEDGMENTS

We thank Dr M.Pope, Dr J.Weiss, Professor M.Greaves, Dr B.Jones, Mr P.Hamilton and Dr A.R.Morris for their help in the investigation of these two cases.

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Obituary

Dr I.B. Sneddon CBE, MD, FRCP

Dr I.B.Sneddon, formerly consultant dermatologist at the Royal Hallamshire Hospital, Sheffield, died on 10 October 1987, aged 72.

Ian Bruce Sneddon was born in Sheffield on 6 March 1915, the only son of a Scottish general practitioner who had settled in the city. His father died when Ian was a schoolboy. He was educated at St. Anselm's School, Bakewell, followed by Uppingham School and Sheffield University. He graduated MB, ChB in 1937 and after house posts in medicine and surgery at the Royal Infirmary, he was appointed registrar to the Skin Department in 1938 and was also registrar in general medicine to Dr (later Lord) Platt.

As he was in the RNVR he was called into the Royal Navy at the outbreak of war and after serving 2 years at sea he was made a skin and medical specialist. Part of the rest of his naval service was spent in the Pacific, and while in Australia he made a film with C.A.Clarke on nutritional neuropathy in prisoners of war on their release. He retained contact with his friends in Australian dermatology for the rest of his life. He was demobilized in 1946 having reached the rank of Surgeon Lt. Cdr.

He returned to Sheffield as registrar to the Skin Department and during the same year worked for a time as registrar to Dr Goldsmith at University College Hospital, London. It was his original intention to take up general medicine, but he was persuaded by Dr Renwick Vickers to join him as consultant in the Skin Department. Together they set the high standards of a department in which patient care and student

teaching took pride of place. He retained his interest in general medicine and was very much a physician in diseases related to the skin.

In the same year, 1946, he met and married Joan who was not only a great support for the rest of his life, but also shared his enthusiasm for medicine and became co-author of some of his later papers in which her own speciality of psychiatry played a part.

He was a popular and enthusiastic teacher with great interest in and concern for young people; as a result of this he gave considerable time to the Medical School, being Honorary Clinical Lecturer from 1946 and Clinical Dean from 1950 to 1968. During the latter appointment he initiated a tutorial system for students so that they should have someone to turn to for advice. When he retired, the University of Sheffield rewarded him for his many services to it with the honorary degree of MD.

Throughout his career and even after he retired from hospital practice he wrote a constant stream of clinical papers, all remarkable for their shrewd observations and clarity of presentation. That which gave him the immortality of an eponymic title was his account with Wilkinson of 'Subcorneal pustular dermatosis' (1956). This was only one of 100 such publications, in addition to books such as 'Practical Dermatology' first published in 1964, and numerous chapters in books including Rooke's 'Textbook of Dermatology'.

In great demand as a lecturer, he was the Dowling Club Orator in 1957, the Watson Smith Lecturer at the Royal College of Physicians, London, in 1963 and the Thom Bequest Lecturer at the Royal College of Physicians of Edinburgh in 1968. He made numerous visits to dermatological centres overseas, was Visiting Professor at the University of Texas in 1979 and delivered invited lectures all over the world from New Zealand to Sweden. Corresponding member of numerous foreign Dermatological Societies, he was also an honorary member of the Danish and of the South African Societies.

He was President of the British Association of Dermatologists for its 50th Annual Meeting in 1970, an occasion which was celebrated with a dinner and ball at the Cutlers' Hall. Subsequently, he was made an Honorary Member of the Association. In 1974, to the pleasure of all his friends, he was awarded the CBE.

He was a regular attender at the Dermatological Section of the Royal Society of Medicine and despite the distance involved occasionally brought patients for demonstration. In 1981 he was President of the Section and in 1986 received the rare distinction of Honorary Membership. At various times he was President of the North of England Dermatological Society and of the Sheffield Medico-Chirurgical Society.

Throughout his career he took a keen interest in the training of young dermatologists and used his influence to correct the imbalance in numbers between consultant and training posts. Numerous consultants throughout the United Kingdom have sat at his feet at some time in their training.

He retired from hospital practice in 1980, but continued in private practice until the end of his life.

His main interest outside medicine was sailing, in his younger days in dinghies and in recent years in his 10 tonner which he kept at Cowes. Many though his achievements were, one of which he was most proud was passing the examination for the Yachtmaster's Certificate in 1987.

He was wise, witty and good company; the sort to whom others turned for advice and help, in which he never failed. His family, friends and patients will sadly miss him. He is survived by his wife, Joan, three daughters and two sons, one of whom is carrying on the family tradition of medicine.

R.E.C.

Obituary

Dr Isaac Muende, BSc (Hons), MBBS, FRCP

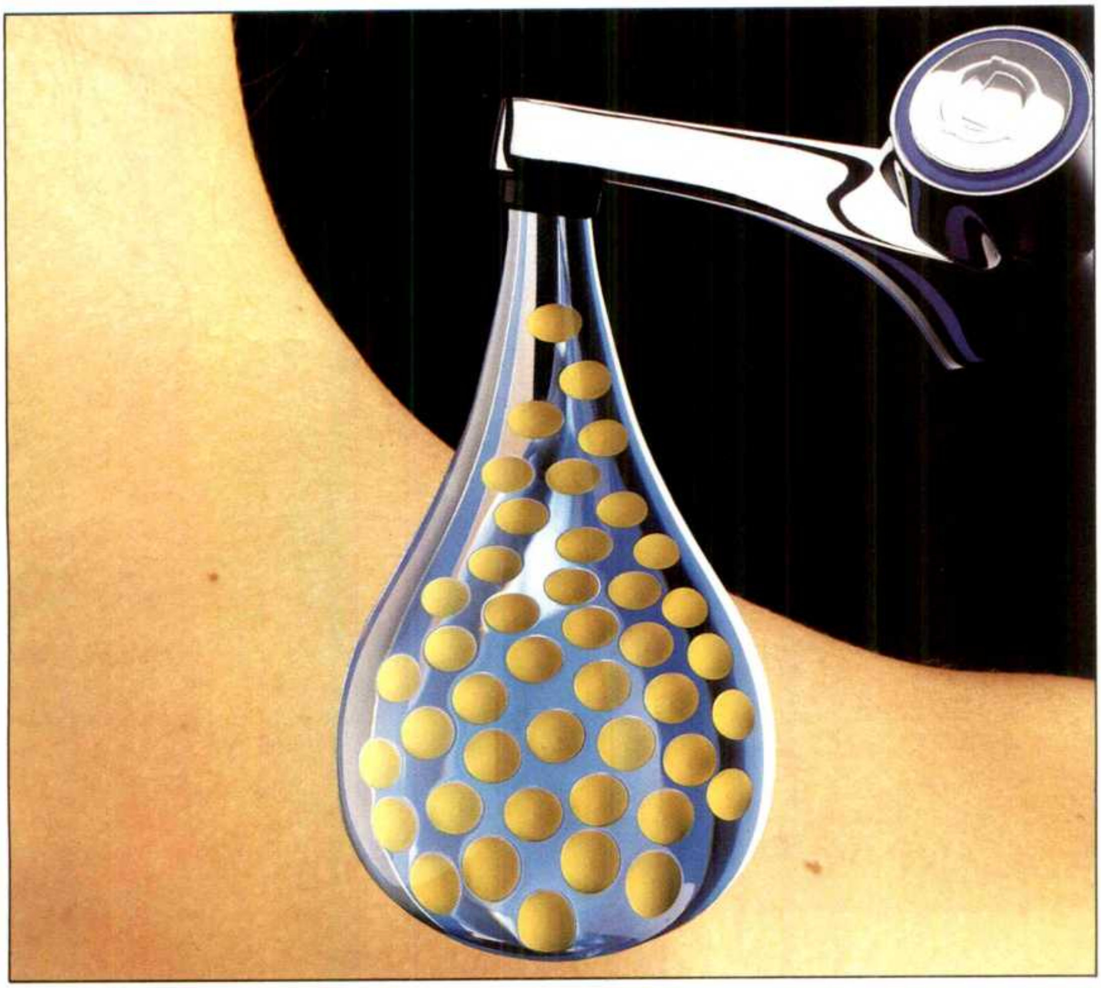
Dr Isaac Muende, formerly consultant dermatologist to St John's Hospital for Diseases of the Skin, Edgware General and Willesden General Hospitals, died on 29 September 1987, aged 86 years.

A modest and charitable man, he was a brilliant teacher, and many generations of dermatologists benefitted from his knowledge and ability. He trained at King's College and Charing Cross Hospital, and won the Radcliffe Crocker Travelling Scholarship and gold medal in 1929 which enabled him to work in Paris, Vienna and Zurich. On his return he set up the first histopathology laboratory at St John's Hospital for Diseases of the Skin, in 1930, but gave up his pathology post in 1948. While there, he wrote a Practical Handbook of Pathology of the Skin—one of the first of such textbooks on the skin to be published in English. He had qualified as an engineer before taking up medicine, was good with his hands and an able technician.

Dr Muende had a most efficient card index system, which he used constantly. When communicating with a general practitioner, he would take the opportunity of asking about other patients whom he had seen in the past, and keep his records up to date. It was through sheer hard work and good communication that this excellent dermatologist built up a huge private practice. He regarded chess and similar games as a waste of time and felt that the practice of good dermatology should fill his working life. He founded the I. Muende Charitable Trust 20 years ago and gave much to charity, not only in money, but in real interest for the people he had helped and with whom he kept in touch.

He was a keen gardener and carpenter and loved his home. His interest in history and archaeology led him to travel widely after his retirement. He maintained his enthusiasm and interest to the last and was still planning trips abroad at the time of his death.

SUZANNE ALEXANDER



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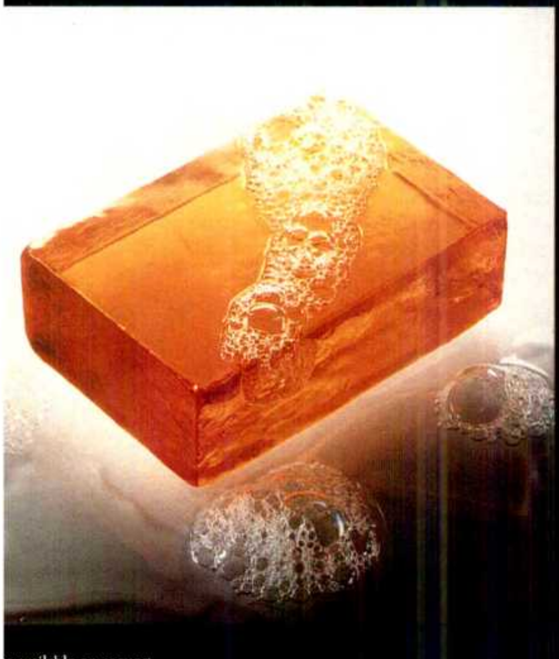

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Correspondence

Dust-like particles: a specific direct immunofluorescence pattern in sub-acute cutaneous lupus erythematosus

MADAM, Sub-acute cutaneous lupus erythematosus (SCLE) is a distinct subset of LE.[1] It is characterized by non-scarring erythematosquamous lesions on sun-exposed areas. Two varieties have been recognized: an annular form with a peripheral colarette and a form with papulo-squamous lesions.[2] Systemic symptoms, of which arthritis is the most frequent, are usually mild. Histopathology shows mild epidermal atrophy, patchy ballooning degeneration of the basal cell layer and a mild and superficial lymphohistiocytic infiltrate.[3] Anti-SSA antibodies and high titre ANA are present in about 60% of cases.[4] In SCLE, direct immunofluorescence (IF) patterns have been described compatible with discoid LE (60%) or with systemic LE (25%).[5] A direct IF pattern specific for SCLE has not been described. We report here the occurrence of such a specific direct IF pattern. It consists of very fine granular, dust-like particles (DLP) of IgG scattered through the epidermis and the cellular infiltrates, with or without fine granular staining of the epidermal nuclei.

Direct IF investigations were done on 51 biopsies from lesional skin and on 14 biopsies from normal skin from 35 SCLE patients. Controls included 29 biopsies from 27 DLE patients and seven biopsies from six SLE patients. The diagnosis of SCLE was based on the clinical features, history and clinical course, and confirmed by histopathological and serological data. Antibodies against IgG, IgA, IgM, IgE, albumin, fibrinogen, C_{3c}, C_{3d} and C_{1q} were used. Microscopic examination was done with narrow band-epi-illumination.[6] Specific blocking studies were performed by preincubation of the sections with unlabelled anti-IgG antibody. This resulted in a marked inhibition of the staining. ANA and anti-SSA antibodies were determined according to standard methods in 27 sera from 27 patients. Statistical analysis was performed using the chi-square test.

A distinct pattern of inter- and intracellularly located very fine, dust-like particles (DLP) of IgG was found throughout the basal layers of the epidermis, the subepidermal region and the dermal cellular infiltrates (Figs 1 and 2). This was seen in 16 (31%) biopsies from 12 (34%) SCLE patients. A correlation between the presence of DLP and the clinical subsets of SCLE could not be established. DLP were not seen in biopsies from DLE or SLE (Table 1). The DLP were positive with anti-C_{1q} in 62%, more obvious in the dermal infiltrates than in the epidermis. Staining with anti-C_{3c} and -C_{3d} was found in two biopsies. In addition a very fine granular staining of the epidermal nuclei was observed in about 50% of the biopsies positive with DLP (Fig. 3). IF patterns compatible with DLE or SCLE were found in DLP negative biopsies of SCLE. Fibrillar deposits of fibrin along the epidermal basement membrane were seen in 75% of the biopsies, localized where ballooning degeneration was present. DLP were seen in only one of the 14 biopsies from normal skin.

The correlation between the presence of DLP and the presence of anti-SSA-antibodies or ANA was not significant (Table 2). The presence of dust-like particles can easily be overlooked because the often patchy deposits of very small granules suggest technical artefacts, e.g. due to insufficient washing or drying of the sections before mounting. In cases of doubt the IF processing should, therefore, be repeated. Another source of misinterpretation may be the non-specific fine granular epidermal staining that can be observed in many spongiotic diseases due to fluid exudation (Fig. 4). In these cases, irregular deposits are located only intercellularly and are positively stained not only with IgG, but also with other antisera, especially anti-fibrinogen and anti-albumin. Fine granular epidermal nuclear staining and DLP over the infiltrates are absent in these cases. In our opinion these circumstances have led to the prevailing opinion that differentiation between SCLE and the other subsets of LE is not possible by the direct IF technique.

Our results indicate that the occurrence of DLP is highly specific for SCLE within the LE group, but that the diagnostic sensitivity is low, (30%). This implies that the value of direct IF is limited in this field. Histopathology and serology remain necessary to assess the clinical diagnosis in SCLE and other subsets of LE.[7]

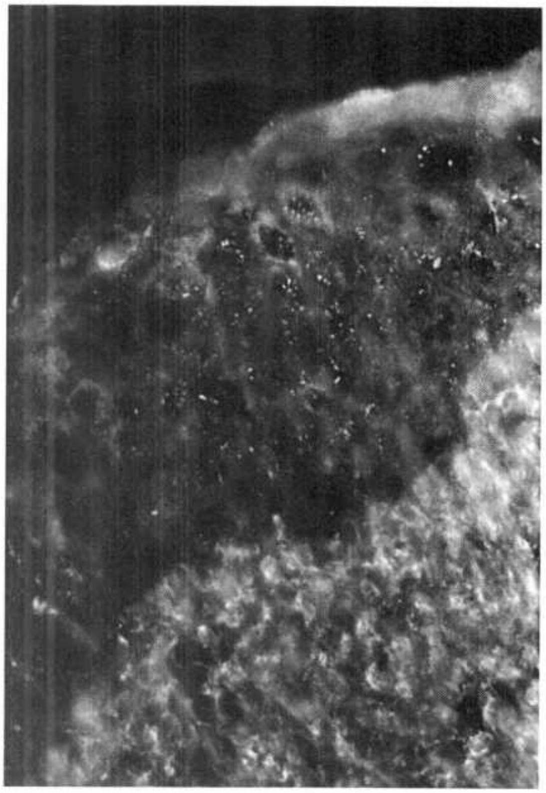

FIGURE 1. SCLE. Dust-like particles of IgG scattered through the epidermal and subepidermal region. (Original × 400).

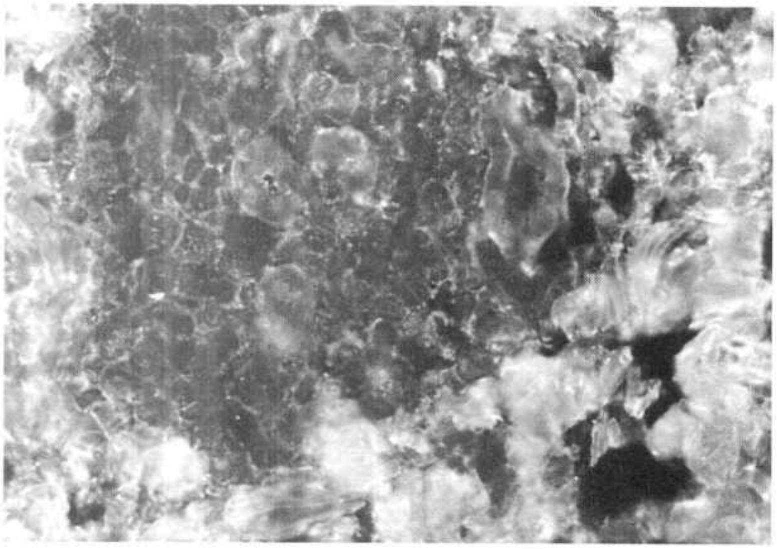

FIGURE 2. SCLE. Dust-like particles throughout a dermal infiltrate. (Original × 400.)

TABLE 1. Direct IF results in SCLE, DLE and SLE

Diagnosis	No. of patients	No. of biopsies	No. of biopsies with positive IF for: SCLE[1]	No. of biopsies with positive IF for: DLE/SLE[2]
SCLE				
Annular	15	22	7	8[3]
Papulo-squamous	20	29	9	10
DLE	27	29	—	22
SLE	6	7	—	6

[1] SCLE: presence of DLP

[2] DLE/SLE: presence of granular or bandlike staining of the epidermal basement membrane.

[3] In eight of the 18 biopsies only weakly positive staining.

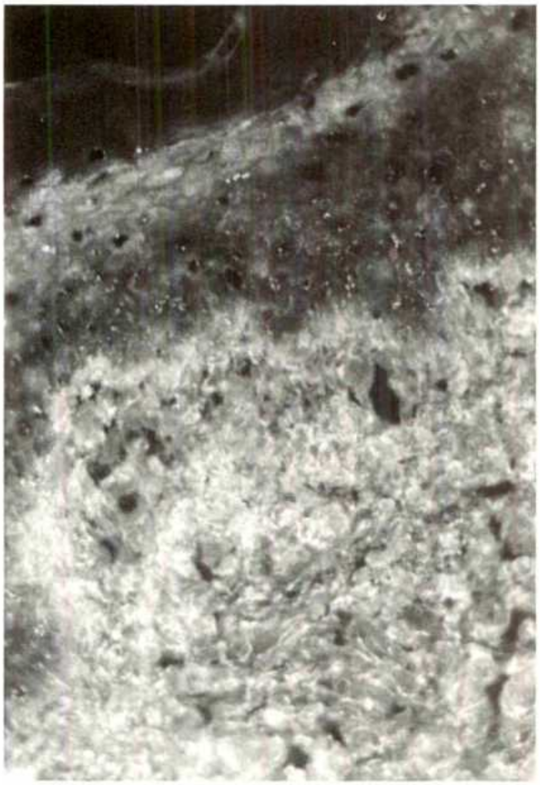

FIGURE 3. SCLE. Fine granular nuclear staining with anti-IgG. (Original × 250.)

TABLE 2. Relation between the incidence of DLP-positive IF and ANA and SSA antibodies in 27 SCLE patients

ANA

DLP	+	−
+	5	4
−	7	11

SSA

DLP	+	−
+	5	4
−	5	13

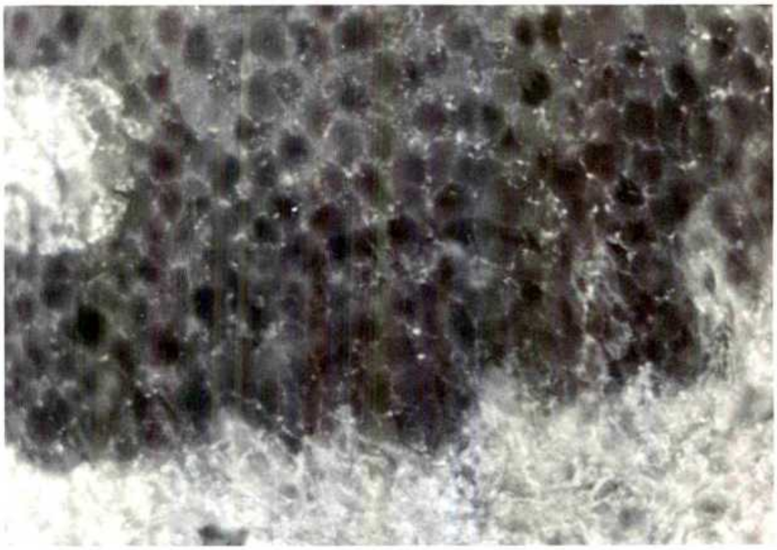

FIGURE 4. Erythema exudativum multiforme: non-specific, intercellular staining with anti-IgG. (Original × 400.)

The nature of the DLP is not clear. There appears to be no significant correlation between the incidence of DLP and that of ANA or anti-SSA antibodies. This does not exclude the possibility that the DLP represent IgG bound nuclear antigens. In this respect it is remarkable that C_{1q} is far more frequently found on the deposits than C_3. This points to an early step in the activation of the complement cascade via the classical pathway and might imply that DLP are *in situ* formed immune complexes. Damage by UV radiation may cause leakage of nuclear antigens out of the nuclei, with subsequent binding to specific IgG antibodies. It may also cause increased permeability for IgG, but not for complement, which has a higher molecular weight, leading to the *in vivo* staining of epidermal nuclei and subsequent leakage of the antigen-IgG complexes.

Department of Dermatology,
Academic Hospital, Free University,
De Boelelaan 1117, 1081 HV Amsterdam
The Netherlands

C. NIEBOER
Z. TAK-DIAMAND
H.E. VAN LEEUWEN-WALLAU

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Congenital poikiloderma: treatment with etretinate

MADAM, We recently described a 6-year-old girl with congenital poikiloderma (Rothmund-Thomson syndrome) who developed multiple warty keratoses on the feet, knees and elbows.[1] Dysplastic change within these lesions was demonstrated by histological, ultrastructural and cell kinetic methods. The lesions recurred after treatment by curettage, and topical 5-fluorouracil cream was ineffective.

Because of increasing disability, treatment with etretinate was started at a dose of 1 mg/kg/day. After 3 months of treatment, there was no obvious clinical improvement. By 6 months, however, the lesions had cleared almost completely (Figs 1a and b) and she was able to resume normal activites. Three months after discontinuing etretinate, the patient remained virtually clear and there was no sign of recurrence of the

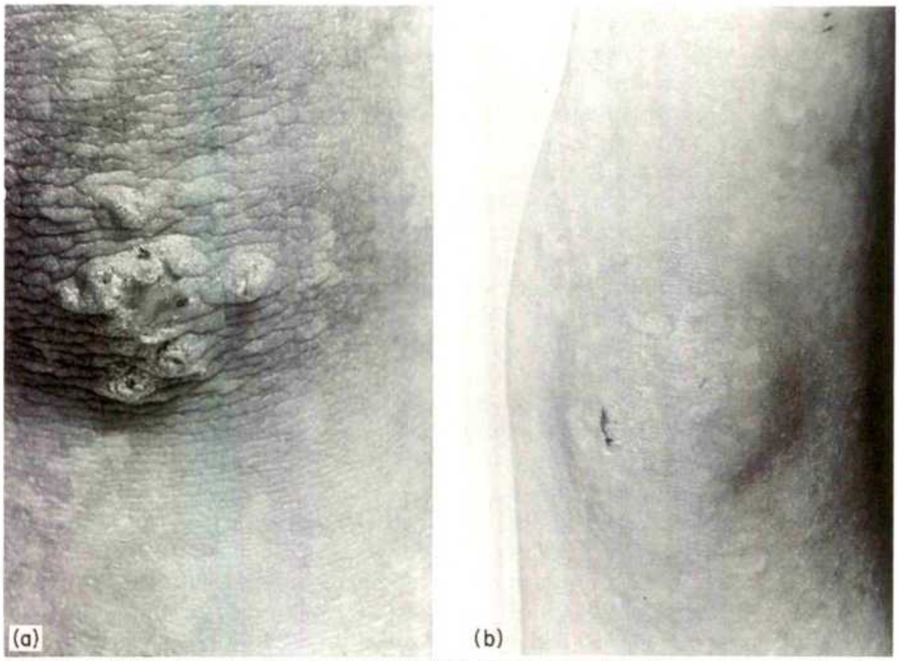

FIGURE 1. Right knee (a) Pre-treatment: Multiple keratoses. (b) Post treatment: Complete clearance of lesions.

keratoses. The usual retinoid side effects of mucosal dryness and cheilitis were observed during treatment, but were well tolerated.

This would appear to be a further example of the ability of the retinoid agents to cause the regression of dysplastic epidermal change, and is consistent with the findings of previous studies relating to the treatment of pre-malignant disease with etretinate.[2] We suspect that the improvement obtained in our patient may only be temporary, but the clinical benefit has been very worthwhile.

Department of Medicine (Dermatology),
University of Wales College of Medicine,
Heath Park,
Cardiff CF4 4XN, U.K.

D. SHUTTLEWORTH
R. MARKS

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Cell-mediated immunity in cutaneous tuberculosis

MADAM, There has recently been a steep increase in the incidence of cutaneous tuberculosis in India. This coincides with a continuing decline in the incidence of pulmonary tuberculosis, and is also seen in other parts of the world.[1,2] It has been suggested that cell-mediated immunity plays a decisive role in determining the clinical manifestations of tuberculosis and we, therefore, decided to investigate cell-mediated immunity in 20 patients with cutaneous tuberculosis. The diagnosis was established according to the criteria of Wilkinson[2] as re-infection (13 patients) or reactivation (seven patients).

Lymphocyte subpopulations were quantitated by the standard indirect immunofluorescent technique. CD4 helper inducer subsets and CD8 cytotoxic suppressor subsets were quantitated using anti-human antibodies (Dakopatts, Denmark). Total and differential white cell counts were carried out on heparinized blood samples to obtain both absolute numbers and percentages of lymphocytes and lymphocyte subsets.

The Mantoux test was performed by injecting 0·1 ml of tuberculin (10 tuberculin units) (Span Diagnostic, Surat, India). The test was read at 48 h and the induration along the longest diameter at the site of injection was graded as 0–4 mm negative, 5–10 mm +, 11–20 mm + + and greater than 20 mm + + +. We also carried out the leukocyte migration inhibition test according to the standard method of Myrbang.[3] Leukocyte rich plasma was collected from heparinized blood and a concentration of 10^7 leukocytes per ml placed in microcapillary tubes which were plugged at one end with inert clay and centrifuged at 600 *g* for 5 min. These capillaries were then cut at the cell interface and fixed with silicone wax in perspex chambers. The antigen used in this case was tuberculin in a 1 : 1000 dilution. After 20 h incubation at 37°C, the area of migration of white cells exposed to tuberculin was compared with that of cells exposed only to tissue culture medium. A migration index of more than 0·8 was taken as significant. Student's *t*-test and the chi-squared test were used for statistical analysis, where appropriate.

There were no differences between the two groups of patients and a group of healthy controls with regard to total lymphocyte counts, total T lymphocytes, CD4 and CD8 positive cells and CD4/CD8 ratio. Mantoux test readings in the two types of tuberculosis showed a response of 24·2 ± 10·2 mm in the re-infection group compared with 19·3 ± 7·8 mm in the reactivation group. The difference between these figures was not significant. Similarly there was no statistically significant difference between the mean migration index of the re-infected, reactivated and healthy control groups.

Thus our study has failed to demonstrate any significant differences between circulating T lymphocytes and lymphocyte subsets, leukocyte migration inhibition on exposure to tuberculin, and strength of Mantoux test reactions between re-infection and reactivation tuberculosis.

Department of Dermatology and Venereology
Maulana Azad Medical College
New Delhi 110 002, India

V.N.SEHGAL
P.AHUJU
V.K.SHARMA

Continuous erythema multiforme clearing on dapsone

MADAM, Continuous erythema multiforme (EM) has been defined as the presence of persisting target lesions on the hands and feet and widespread, recurrent papulo-necrotic or vesicular lesions. This condition is apparently rare with only three cases reported in one series of 26 patients with EM.[1] As the lesions tend to be widespread, continuous EM constitutes a prolonged and distressing condition. We describe a patient with continuous EM precipitated by herpes virus infection. Therapy with acyclovir and systemic corticosteroid was unsuccessful, but the lesions cleared completely on dapsone, without relapse.

A 33-year-old Pakistani man developed a widespread pruritic eruption 10 days after the onset of a cold store on the lip in February 1986. Previously, he had had uncomplicated herpes labialis recurring at 3–4 monthly intervals, and his only medication was oxytetracycline taken for 2 years for moderate acne. Examination showed characteristic target lesions of EM on the hands, arms and legs, and vesiculonecrotic and bullous lesions on the trunk and face. Enveloped herpes virus was visualized on electron microscopy from a lip lesion, but viral culture was sterile. The following investigations were normal or negative: FBC, biochemical profile, CH_{50}, C_3 and C_4, circulating immune complexes, ANA and viral antibody titres. A skin biopsy of a lesion on the trunk was compatible with bullous EM. Direct immunofluorescence of lesional and non-lesional skin and indirect immunofluorescence were negative. Treatment with intravenous acyclovir and oral prednisolone 60 mg daily proved unsuccessful and the patient was never free of lesions, which remained widespread until June 1986 when dapsone 100 mg daily was commenced. The development of new lesions ceased after 2 weeks and his skin became totally clear after 1 month. There has been no relapse of either EM or herpes labialis despite discontinuing dapsone for 1 year.

A dramatic response on dapsone of a condition which had previously shown no sign of remission strongly suggests a therapeutic efect of the drug, although spontaneous resolution cannot be entirely excluded. Further reports of the use of dapsone for continuous EM would help to asses its therapeutic merit.

The Skin Hospital
George Road, Edgbaston
Birmingham B15 1BR, U.K.

P. DUHRA
C.J. PAUL

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Sub-acute cutaneous lupus erythematosus and gluten sensitive enteropathy

MADAM, I would like to report a case of sub-acute cutaneous lupus erythematosus with gluten sensitive enteropathy. Although there have been a few previous reports of malabsorption and protein losing enteropathy associated with systemic lupus erythematosus (SLE),[1] to my knowledge, this is the first report of sub-acute cutaneous lupus erythematosus (SCLE) associated with gluten enteropathy. This may be more than a chance occurrence, particularly as both conditions are associated with an increased incidence of HLA DR3.

A 53-year-old woman first presented to a general practitioner with a history of daily diarrhoea, a poor appetite and weight loss of 14 lbs. Investigations over a period of 4 months revealed a normal full blood count, serum albumin 25 g/l (normal range 35–53 g/l), total serum protein 53 g/l (normal range 60–80 g/l), but otherwise normal liver function tests.

Other investigations, including a barium meal, barium enema, endoscopy and sigmoidoscopy were normal. A jejunal biopsy, however, revealed sub-total villous atrophy.

Subsequently, it was found that she had a low serum folic acid level < 1 ng/l (normal range 2–14 ng/l) and a Schilling test showed a low ratio of unbound vitamin B_{12} excretion, compatible with malabsorption. In view of these investigations, a diagnosis of gluten sensitive enteropathy (GSE) was made. She was started on a gluten-free diet and gained weight, with improvement in her gastro-intestinal symptoms.

She presented to the dermatology clinic 12 months later with a history of skin lesions, mainly on the face and forearms. She was otherwise in good health and was still maintained on a gluten-free diet.

Examination at that time revealed erythematous annular and gyrate lesions on the face, neck and forearms.

Investigations of her skin disorder revealed normal complement levels, negative anti-nuclear factor and latex globulin tests, but positive anti-ENA antibodies and anti-Ro antibodies.

Histology of her skin revealed prominent basal cell melanin pigmentation with minimal focal chronic inflammation and overall apparent atrophy of the skin. Direct immunofluorescence of a skin lesion was negative. A diagnosis of SCLE was made on the basis of the clinical appearances and positive anti-Ro antibodies. The lesions healed rapidly without scarring over the winter months. The only topical treatment was a low strength fluorinated steroid cream.

There have been three previous reports of a protein losing enteropathy associated with systemic lupus erythematosus.[1]

In addition, a patient was recently shown at the British Association of Dermatologists as having sub-acute cutaneous lupus erythematosus and malabsorption. In this particular case, a jejunal biopsy was normal.[2] It is interesting to note that patients with SCLE, who have positive anti-Ro antibodies have a high incidence of HLA DR3.[3] HLA DR3 is also a marker for gluten sensitive enteropathy.[4]

This is the first case, to my knowledge, of sub-acute cutaneous lupus erythematosus associated with gluten sensitive enteropathy. It may be that this is purely coincidental. However, in view of the previous reports of protein losing enteropathy and malabsorption in systemic LE, and also the increased frequency of the same HLA marker in both SCLE and gluten sensitive enteropathy, there may well be a genuine association.

Perhaps it would be interesting for centres which see many cases of sub-acute cutaneous LE to look specifically for evidence of malabsorption and gluten sensitivity.

Singleton Hospital,
Sketty, Swansea SA2 8QA, U.K.

D.L. ROBERTS

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International Society for the Study of Vulvar Disease—Progress Report

MADAM, In a recent Comment article (*British Journal of Dermatology* 1986; 115: 647) the proposed nomenclature of non-neoplastic epithelial disorders of vulval skin and mucosa, which was then under discussion by a sub-committee of the International Society for the Study of Vulvar Disease (ISSVD), was outlined.

At the 9th Congress of the ISSVD in September 1987, the proposal of the International Society for Gynecological Pathologists (ISGYP) was that three groups should be used:

(a) lichen sclerosus (lichen sclerosus et atrophicus);
(b) squamous hyperplasia, not otherwise specified (NOS);
(c) other dermatoses.

The final form ratified by the ISSVD is:

(1) Lichen sclerosus;
(2) Squamous cell hyperplasia (formerly hyperplastic dystrophy);
(3) Other dermatoses.

Mixed epithelial disorders may occur. In such cases it is recommended that both conditions be reported. For example, lichen sclerosus with associated squamous cell hyperplasia (formerly classified as mixed dystrophy) should be reported as lichen sclerosus and squamous cell hyperplasia. Squamous cell hyperplasia with associated vulvar intraepithelial neoplasia (formerly hyperplastic dystrophy with atypia) should be diagnosed as vulvar intra-epithelial neoplasia.

Squamous cell hyperplasia is used for those instances in which the hyperplasia is not attributable to another cause. Specific lesions or dermatoses involving the vulva (e.g. psoriasis, lichen planus, lichen simplex chronicus, *Candida* infection and condyloma acuminatum) may include squamous cell hyperplasia, but should be diagnosed specifically and excluded from this category.

This classification will be published in the *Journal of Reproductive Medicine*, and will go forward to the next meeting of the appropriate sub-committee of the ISGYP. Thus, we anticipate that agreement between the interdisciplinary ISSVD and the ISGYP will be achieved, which is gratifying on academic and clinical grounds. The new nomenclature appears serviceable and more acceptable than the old to dermatologists.

St. Mary's Wing
Whittington Hospital
London N19 5NF, U.K.

C.M.RIDLEY

Failure of neutrophils to migrate into psoriatic lesions

MADAM, The recent observation by Lammers and van de Kerkhof (*British Journal of Dermatology* 1987; **117:** 541) of the total inhibition of leukocyte migration into psoriatic lesions after local LTB_4 application is indeed intriguing. It fits nicely with the notable absence of neutrophils at sites of chronic inflammation and with the paucity of these cells in the epidermis of chronic psoriatic lesions despite the fact that large quantities of potent chemotactic factors have been demonstrated in psoriatic lesions and scales.[1,2] As a possible explanation of their observation, the authors suggest that LTB_4 is metabolized faster and thus inactivated more rapidly in lesional skin than in uninvolved psoriatic skin.

While this is a good hypothesis, I would like to point out that, on the basis of published evidence, there are several other possible explanations for the observed phenomenon.

All chemotactic factors, including LTB_4 inhibit or deactivate neutrophils at high doses *in vitro*.[3] Since levels of LTB_4 and other chemotactic factors are increased in lesional skin and psoriatic scales,[1,2] their concentration might be sufficient to prevent the accumulation of neutrophils. Application of additional LTB_4 would even enhance this process.

Mononuclear cells are known to produce cytokines such as leukocyte and macrophage migration inhibitor factors (LIF and MIF).[4] Furthermore, inhibitors of leukocyte migration have been demonstrated in normal serum, in the serum of patients with chronic inflammatory diseases and in granulocyte extracts.[5-8] It is possible that such factors are also present and functional in psoriatic lesions and that they accumulate in the scales.

We have in fact demonstrated such activity in psoriatic scales.[9] It inhibits leukocyte migration towards LTB_4, and also towards other chemotactic factors (Czarnetzki *et al.*, unpublished). The material is heat labile and was found to consist of several molecular species when analysed by column chromatography. While this material can also be demonstrated in normal callus, more of it would be expected to be present in psoriatic scales. This material is one of the most likely factors to have been responsible for binding and inactivating the LTB_4 applied to lesional skin in Lammers and van de Kerkhof's experiments.

Department of Clinical Research, Dermatology
F. Hoffmann La Roche
CH 4058 Basel, Switzerland

BEATE M. CZARNETZKI

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Fungal infection in renal transplant patients

MADAM, We read the article 'Fungal infection after renal transplantation' (*British Journal of Dermatology* 1987; 117: 585) with interest. As part of a larger study of 121 consecutive renal allograft recipients attending our transplant clinic for routine follow-up, patients were examined for evidence of fungal infection. Skin scrapings were taken from clinically suspicious lesions and the scrapings were examined by microscopy and culture.

Ten patients were found to have a fungal skin infection, four with *Tinea rubrum* and six with *Malassezia furfur*. The presence of a fungal infection was not significantly associated with age, sex, sun excess, duration of allograft, drug regimen or the presence of viral warts or dysplastic skin lesions. There was a statistically significant association with skin type; those who tanned easily were more likely to have a fungal skin infection than those who burned on sun exposure ($P < 0{\cdot}01$). The reason for this is uncertain, but may reflect a racial susceptibility to fungal infection that is exaggerated by the systemic immunosuppression.

Dermatology Department,
Royal Postgraduate Medical School,
Hammersmith Hospital,
Ducane Road, London W12 0HS, U.K.

JANET MCLELLAND
A.C. CHU

British Journal of Dermatology (1988) **118**, 735.

News and Notices

International Update in Dermatology and Dermatopathology

An International Update in Dermatology and Dermatopathology, organized jointly by the Pathological Society of Great Britain and Ireland and the British Society for Dermatopathology, will be held in Brighton, U.K. from 2–5 September 1989. Topics will include: appendage tumours, connective tissue neoplasms, melanocytic tumours, inflammatory dermatoses, lymphoid infiltrates, immunologically-mediated skin diseases, vasculitides, and epidermolysis bullosa and other bullous dermatoses. There will also be two slide seminars. For further information please contact: Dr P.H.McKee, British Association of Dermatologists, 6 St. Andrew's Place, Regents Park, London NW1 4LB, U.K.

Towards a Molecular Basis of Skin Photobiology

A Harden Satellite meeting, Towards a Molecular Basis of Skin Photobiology, supported by the Biochemical Society and the British Photobiology Society, will be held in London, U.K. on 16–17 December 1988, preceding a 1-day meeting on Free Radicals and Disease to be held on 19 December 1988. For further information contact: Dr Antony Young, Photobiology Unit, Institute of Dermatology, University of London, Renfrew Road, London SE11 4TH, U.K. Telephone 01-793 0387.

Cancellation of Clinically Orientated Symposium on Bullous Disorders

Regretfully, the Clinically Orientated Symposium on Bullous Disorders, scheduled for 20–21 January 1989 (as announced in the March issue), has been cancelled.

International Epidermolysis Bullosa Symposium
Alteration in Arrangements

The International Epidermolysis Bullosa Symposium will now be held on Thursday and Friday, 19–20 January 1989, in London U.K., and not on 18–19 January 1989 as announced in the March issue. This meeting will be of interest to members of all the different disciplines involved in the management of epidermolysis bullosa sufferers. Invited speakers will lecture on advances in research and on wide-ranging clinical topics. There will also be free communication sessions, and the proceedings will be published. For further information and abstract forms contact: Mrs Mary Freeland, D.E.B.R.A., Suite 4, 1 Kings Road, Crowthorne, Berkshire RG11 7BG U.K. Telephone 0344 771961. Closing date for receipt of abstracts, 14 October 1988.

British Journal of Dermatology (1988) **118,** 736.

Errata

(Vol. 118, Number 1)

Lichen sclerosus et atrophicus and autoimmunity—a study of 350 women. R.H.Meyrick Thomas *et al.* 1988; **118:** 41, Table 2, Autoimmune related diseases in patients with LSA, should read as follows:

	Patients	
	No.	%
Alopecia areata	32	9
Vitiligo	21	6
Thyrotoxicosis	10	6
Hypothyroidism	12	
Pernicious anaemia	6	2
Diabetes mellitus (insulin dependent)	3	1
Bullous pemphigoid	2	1
Systemic lupus erythematosus	1	
Primary biliary cirrhosis	0	

(Vol. 118, Number 2)

K.E.Virvidakis, J.D.Brokalakis, P.N.Singhellakis and T.D.Mountokalakis. Effect of PUVA on intestinal calcium absorption. 1988; **118:** 219–221. The first paragraph in the Methods section on page 219, second line down, should now read:

Patients were divided into two groups: Group 1 included seven patients, aged 46–75 years, who were treated with oral 8-MOP, 0·6 mg/kg, followed by whole-body exposure to long-wave (320–400 nm) ultraviolet radiation (UVA), 2 h later. Treatment was repeated four times a week with radiation doses starting at 2 J/cm^2 and increasing by 1 J/cm^2 every second session. The total dose of UVA in each patient over 14 days was 28 J/cm^2.

Also, the first paragraph in the Results section on page 220, first line down, should now read:

Patients treated with PUVA for 14 days showed a significant increase in mean a value (± SEM) from 23·8 ± 1·94 to 38·5 ± 3·86 ($P < 0{\cdot}01$) while no significant change in mean a value occurred in the patients exposed to UVA radiation alone (29·0 ± 3·22 and 27·0 ± 3·75, respectively).

British Journal of Dermatology (1988) **118**, 737–744.

South American pemphigus foliaceus: Study of an epidemic in El Bagre and Nechi, Colombia 1982 to 1986

MARY ANN ROBLEDO, STELLA PRADA DE C., DIEGO JARAMILLO AND W.LEON

Dermatology Section, Department of Internal Medicine, University of Antioquia, Medellin, Colombia

Accepted for publication 16 December 1987

SUMMARY

Between 1982 and 1986 we have had the chance to study 21 patients with pemphigus foliaceus of the 'fogo selvagem' type. The patients came from El Bagre and Nechi, rural areas of Colombia with some gold mining.

This is the first outbreak of South American pemphigus foliaceus reported in Colombia.

The majority of the patients were mestizo men, who worked as farmers or miners or both, with an average age of 44. Five patients were relatives. Eleven patients (52%) had mild disease, three (14%) moderate disease and seven (33%) severe disease. During hospitalization, three patients died due to complications or as a result of immunosuppressive treatment. Of the remaining 18 patients, 10 were in remission with treatment, one was in remission without treatment, while no information was available on the remaining seven.

The 'fogo selvagem' type of pemphigus foliaceus (PFS) is a cutaneous disease, characterized by a generalized distribution of bullae, followed by an exfoliative erythroderma. In contrast to other forms of pemphigus it does not affect the mucous membranes. The major histopathological feature is the presence of acantholysis in the superficial layers of the epidermis.[1–4]

The disease has spread in South America through rural and urban epidemics. The first cases were described in 1891 by Alexander Serqueira, but it was only 40 years later that Faviano Silva published his data.[5,6] The disease was first noted in Brazil. From Port Ilheus in Itabuna it was easily spread to the mining states of Gerais and Sao Paulo due to the iron trade. The largest foci in 1912 were Oro Preto and Belo Horizonte. From there it spread to Mato Grosso and the mining triangle of Goais. In 1948, pemphigus reached Buenos Aires via the Parana river and passed on to Northern Paraguay crossing the Velhas river. During the construction of the railroad from Brazil to Bolivia, pemphigus appeared in Bolivia. In 1949 it was observed in Peru and in 1950 in Venezuela.[2,3,7,8] The first cases of this epidemic focus in Colombia were reported during the 'XV Congreso Colombiano de Dermatologia' held in Bucaramanga in 1984.[9]

The epidemic nature of the disease has led to the belief that two factors are involved in the pathogenesis of pemphigus foliaceus, an exogenous causal agent and an endogenous factor (autoantibodies) the former carried by a vector, probably a flying insect.[10-12] Various investigators have tried to demonstrate that the causal agent is a virus, but the results have been varied and not fully confirmed.[13-16] The endogenous factor was demonstrated in 1964 by Beutner and Jordon[17] who used immunofluorescence to show that autoantibodies existed against an intercellular component of the squamous epithelium. Later, Hashimoto and Lever[18] identified that component as the intracellular cement. Immunosuppressive therapy with corticosteroids was introduced in 1960 and has proved to be highly beneficial.[19,20] In more serious cases, other immunosuppressants have been used.[21-23]

We report here 21 patients with pemphigus foliaceus of the 'fogo selvagem' type, all of whom came from El Bagre and Nechi, rural areas of Colombia.

METHODS

Patients

Records of patients who presented between 1976 and 1986 to the Dermatology Section of the San Vincente de Paul Hospital were consulted, and patients diagnosed as having pemphigus foliaceus, and who were living in the El Bagre and Nechi area were selected.

A diagnosis of pemphigus foliaceus was based on the following symptoms: a burning sensation or pruritus or both, and in severe cases cryaesthesia, accompanied by erythematous desquamation and exfoliating lesions with denuded areas and flaccid superficial bullae.

The lesions mainly affected seborrhoeic areas, but they could extend to the whole body in the erythrodermic form. Nikolsky's sign was positive. The patients had a characteristic 'mousy' odour. The diagnosis was confirmed by histopathology and in some positive cases this was supplemented by direct immunofluorescence.

Disease severity was calculated according to the body area affected, using Lund and Browder's rule of nine,[24] as used for burns. Patients were classified as mildly, moderately or severely affected (0–32%, 33–65% and >66% surface area involvement, respectively).

Laboratory examinations and histopathology

A positive diagnosis was established when acantholysis was observed in either the primary subcorneal or the subgranular layer. Direct immunofluorescence was considered positive if deposits of IgG were found in the intercellular spaces of the Malpighian epidermis.

Therapy

Prednisone was used as the basic drug for immunosuppressive treatment. The initial dose was 40, 80 or 120 mg/day, for 4–6 weeks. The dose was then reduced to 40 mg/day for those receiving 80 or 120 mg/day and gradually reduced further to 10–20 mg/day or below. This dose was continued until the disease was either under control or had completely cleared. In some cases methylprednisolone pulses were also used at doses of 0·5 to 1 g given 1 to 4 times and then prednisone reinstituted as before.

An alternative immunosuppressive agent was methotrexate, administered at an initial dose of 25 mg/week for at least 1 month, after which the dose was slowly reduced.

Additionally, isoniazid (300 mg/day) and antiparasitic prophylaxis was administered.

Patients were evaluated clinically before and during therapy so that situations in which corticosteroid therapy was contra-indicated could be avoided.

RESULTS

Between September and August 1986, 21 patients were diagnosed in our clinic as having PFS.

Figure 1 shows the geographical location of the endemic zone with centres in the cities of El Bagre and Nechi, northeast of the Department of Antioquia. Of these patients, 18 (85%) were male and three (14%) were female. They were all mestizos aged between 22 and 82 years, with an average age of 44 years. Five of the patients were relatives. Thirteen patients (62%) were farmers, four (19%) were farmers and miners, two were miners and two (10%) did other types of work. The disease developed over 1 to 24 months, with an average of 7 months.

Figure 2 shows the years in which the diagnoses were made. All 21 patients showed the typical lesions of pemphigus foliaceus with different degrees of severity (Figs. 3, 4 and 5). Eleven patients (52%) had mild disease, three (14%) moderate disease and seven (33%) severe disease.

Diagnosis

Histopathological examination was carried out in 20 of the 21 patients. Direct immunofluorescence was performed in 14 patients with positive results in 13 (92%). Only one patient was

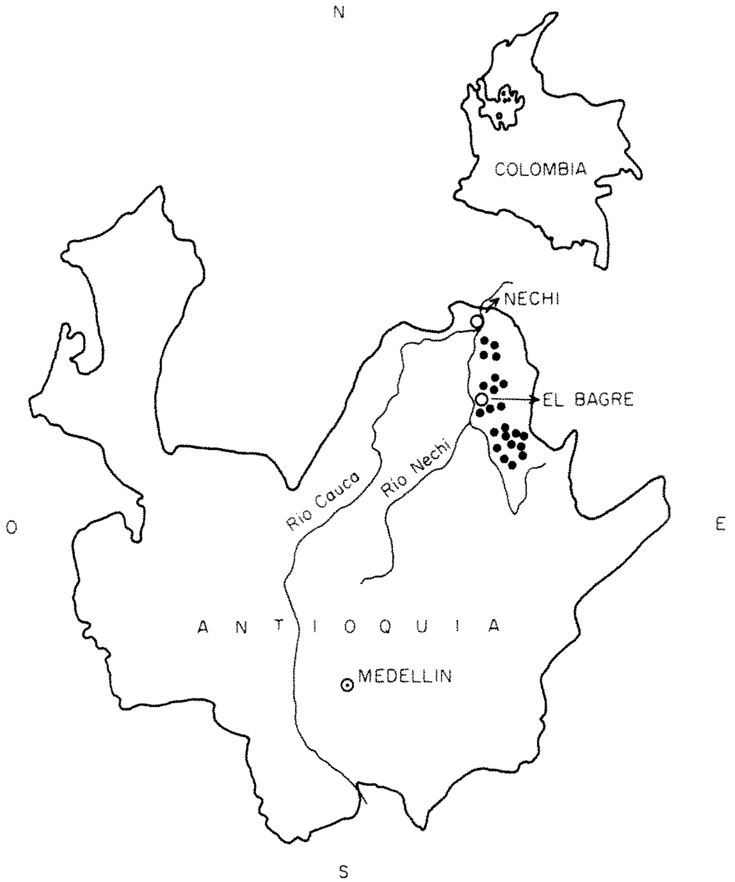

FIGURE 1. Geographical distribution of patients suffering from PFS in the Municipalities of El Bagre and Nechi (*Antioquia*). The dots indicate where each patient came from.

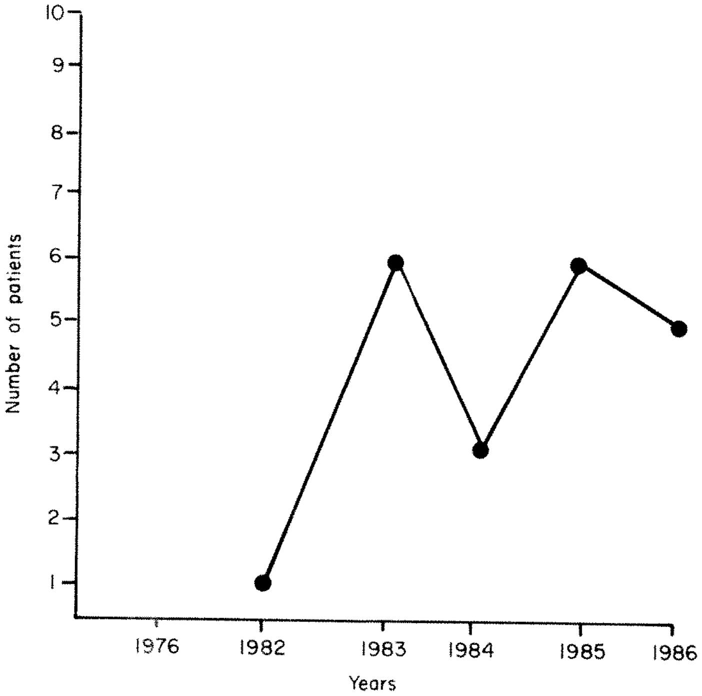

FIGURE 2. Numbers of cases of PFS diagnosed between 1976 and 1986.

considered as a presumptive case. He arrived in hospital with a severe form of the disease and died soon afterwards without immunological or histological confirmation of the diagnosis.

Therapy

The treatment used depended on the severity of the disease (Table 1). Most of the patients showed some improvement 4 to 6 weeks after they began therapy. In August 1986 none of the patients remained in hospital. At that time, one patient, the first to receive treatment, was in remission without drug therapy—10 were in remission on low doses of prednisone and seven had not returned for follow-up.

Three patients died as a result of pemphigus or complications such as renal insufficiency, sepsis caused by *Staphylococcus aureus*, or meningitis caused by *Listeria monocytogenes*.

DISCUSSION

The Colombian literature makes no previous mention of a endemic focus of PFS. Garcia and Cortes[25] reviewed pemphigus admissions to the San Vincente de Paul University Hospital between 1957 and 1969. Altogether they found 34 cases of pemphigus, 19 of these were pemphigus foliaceus of the non-epidemic form. In our region this number has been exceeded as we diagnosed 21 cases in 4 years, suggesting that we are dealing with the epidemic form.

Since the first case was diagnosed in 1982, our unit has received three to six patients per year, but there are more cases in the region who have not received adequate medical treatment. This

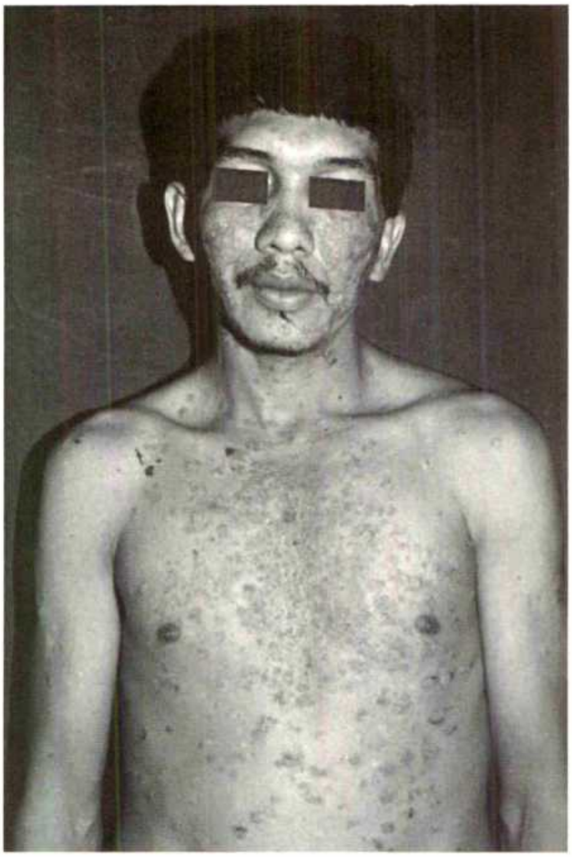

FIGURE 3. A patient with mild pemphigus foliaceus with isolated lesions on the trunk.

suggests that these patients may be the forerunners of a large epidemic extending to Bajo Cauca, Magdelena and Uraba, since these are regions with similar environmental conditions.

In our series males predominated (85%), while females were predominant (65%) in the series reported by Garcia and Cortes.[25] The distribution of the disease between the sexes also varied in the Brazilian literature.[26,27]

In our study in Colombia the average age of the PFS patients was 44 years, while in Brazil it affected people between the ages of 10 and 30 years.[25-28] We found no relationship between the severity of the clinical symptoms and their development, and the age or sex of the patients.

Other authors have already reported relatives infected with PFS, and suggested a genetic element or cohabitation as a predisposing factor.[26,27,29]

In four of our severe cases it was necessary to use methotrexate, and to reduce the dose of prednisone because of poor tolerance. Garcia and Cortes[25] did not need to use methotrexate.

The major question which arises is what has led to the appearance of this variety of pemphigus in El Bagre and Nechi. In 1972 the Colombian government legalized the gold trade and put up the gold price, which led to a progressive increase in gold mining in Antioquia.[30] The 'gold fever' gave rise to a continuous influx of people to the mining centres of northeast Antioquia

TABLE 1. Therapy given to patients with pemphigus foliaceus

	Drug	No. of patients
Mild disease ($n = 11$)	Prednisone 40 mg/day* Prednisone 80 mg/day*	5 6
Moderate disease ($n = 3$)	Prednisone 120 mg/day* Methylprednisolone 'pulses'**	1 2
Severe disease ($n = 6$)	Prednisone 120 mg/day* Methyprednisolone 'pulses'** Methotrexate 25 mg/week	4 2 4

* Initial dose
** 0·5–1 g/dose.

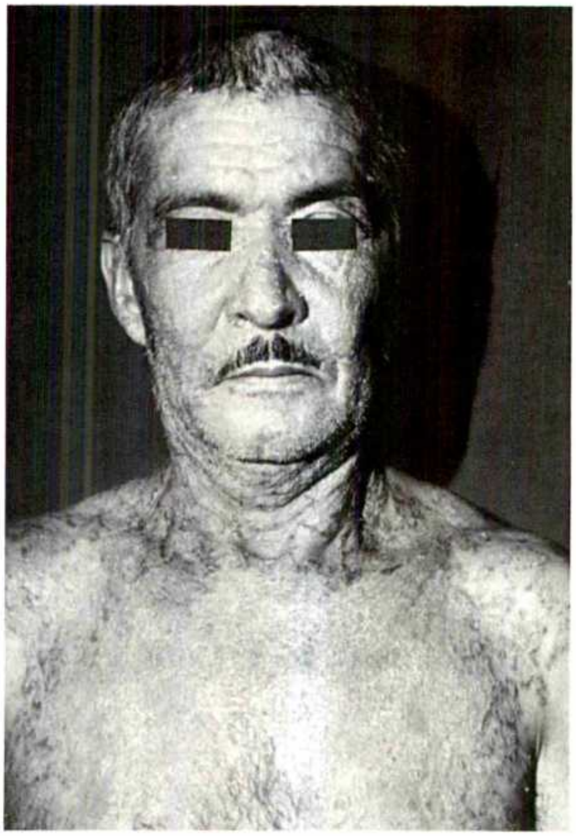

FIGURE 4. A patient with moderate pemphigus foliaceus. Erythematous exfoliating lesions extend over the entire body.

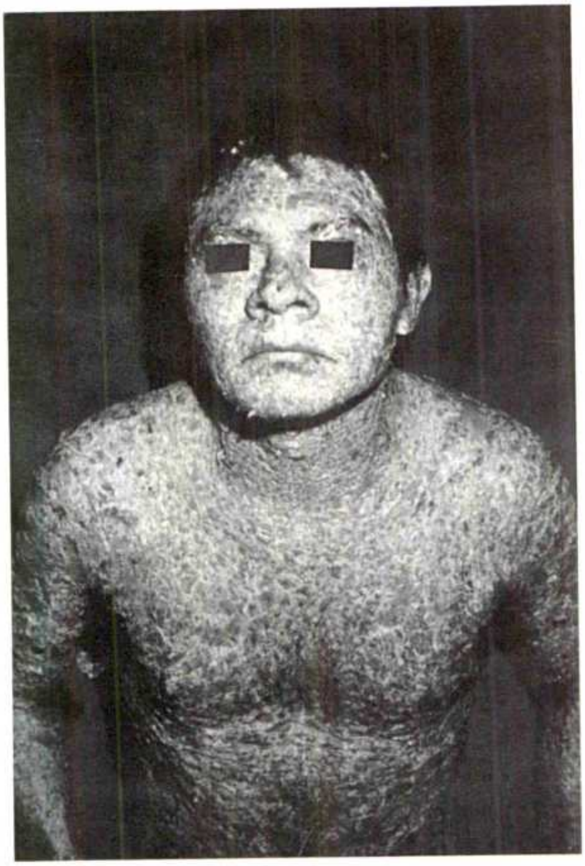

FIGURE 5. A patient with severe pemphigus foliaceus with erythroderma.

(Colombia) resulting in slum settlements, with detrimental effects on the environment and the social and economic progress of the region.[31] This migration included not only Colombians, but also emigrants from neighbouring countries, including Brazil, where the disease is known to be endemic.

These observations are in accordance with those of Aranha Campos who suggested that pemphigus was easily disseminated by diseased individuals or carriers through population movements. Thus, we can see in Colombia a repeat of what happened in the early 1900's in Brazil,[2] and it appears that social, economic and demographic factors influence the spread of the disease.

The epidemic nature of the disease, its apparent spread during population movements, and the possible influence of environmental factors on people working the mines in jungle areas, in our view, confirm the existence of an infectious agent in the aetiopathogenesis of PFS. More specifically an infectious agent may trigger the autoimmune disorder in this disease.

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Dysplastic naevus in non-familial melanoma. A clinicopathological study of 101 cases

J.J.GROB*, LUCILE ANDRAC‡, MARIE HÉLÈNE ROMANO*, D.DAVIN*,
ANNE MARIE COLLET-VILLETTE*, MARIE HÉLÈNE MUNOZ*
AND J.J.BONERANDI*

* Service de Dermatologie Hopital Ste Marguerite, Marseille, and ‡Service d'Anatomopathologie Hopital Timone, Marseille, France

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SUMMARY

In 101 patients with non-familial cutaneous melanoma (CM), melanocytic naevi were counted and classified according to clinical criteria. Only 8% of the patients had very atypical naevi. These atypical naevi were few in number and only one patient exhibited dysplastic naevus syndrome. An histological study was undertaken on the hypothesis that, in a given individual, if the most clinically atypical naevus is not histologically dysplastic it is unlikely that any of the others are. The most clinically atypical naevus in each patient was biopsied. Estimated in this way the prevalence of dysplastic naevi in patients with non-familial CM was only 18%. Comparison of patients with and without dysplastic naevi did not suggest that they constituted two different subsets. An attempt to correlate clinical diagnosis and histological features in this group of patients showed that the diagnosis of dysplastic naevi on the basis of clinical criteria alone is difficult and not reliable.

The term dysplastic naevus is a histological designation of a distinct group of abnormal naevi, which also have clinically atypical features. They were first described by Clark *et al.* in patients with familial cutaneous melanoma (CM).[1,2] In individuals with a family history of CM, the presence of dysplastic naevi constitutes a marker of risk.[3]

Elder *et al.*[4] pointed out the possibility of dysplastic naevi in individuals with non-familial CM. However, the epidemiological value of the dysplastic naevus as a marker of risk of non-familial CM has not been thoroughly investigated.

The present combined pathological and clinical study was undertaken to measure the prevalence of dysplastic naevi in patients with non-familial CM and to investigate the relationship between clinical and histological findings in dysplastic naevi.

Correspondence: Jean Jacques Grob, Service de Dermatologie, Hopital Ste Marguerite, 270 Bd Ste Marguerite BP 29 13277 Marseille Cedex 9, France.

METHODS

Premise

Although certain clinical features are suggestive, diagnosis of a dysplastic naevus cannot be confirmed or eliminated without histological data. From a theoretical point of view, in order to determine whether a given individual does or does not have dysplastic naevi, multiple biopsies should be performed. From a practical standpoint, this is not feasible in a large study. For this reason we systematically biopsied the most clinically atypical naevus in each patient, on the hypothesis that, in a given individual, if the most clinically atypical naevus is not dysplastic, it is unlikely that any of the other naevi are. To check this hypothesis, however, multiple biopsies were carried out in 15 patients.

Patients

Of the patients treated for CM in our dermatology department, 128 patients were asked to participate in the study when they presented for routine follow-up examination. Of these patients, 16 were eliminated because they did not meet one or more of the following criteria: Caucasian, no family history of CM, superficial spreading melanoma (SSM) or nodular melanoma (NM), and resection less than 3 years earlier. Of the remaining 112 patients, 11 refused to undergo biopsy so that 101 were finally included in the study. Patients' ages ranged from 23 to 79 years (mean 47·6 years). Using a working classification of sun reactive skin type,[5] 39 patients were type II, 47 type III, and 15 type IV.

Clinical examination

All patients were thoroughly examined from head to foot including the scalp and genitals. This examination was performed by an experienced clinician and crosschecked independently by a second clinician. Patients were grouped according to the number of melanocytic naevi: (1) on the body as a whole (0, 1, 2 to 5, 6 to 10, 11 to 20, 21 to 50, > 50); (2) in different locations (scalp, face, shoulder and upper arm, forearms, hands, front trunk, back trunk, buttocks, genitals, thighs, legs, and feet); (3) as a function of size (< 5 mm, 5 to 10 mm, 11 to 20 mm, > 20 mm). Although ephelides were obviously not counted, the number of lesions < 5 mm was probably overestimated by unintentional counting of non-naevomelanocytic lesions.

The clinically atypical naevi were considered as suspect dysplastic naevi when they fulfilled at least two of the following criteria[6]: (1) maximum diameter > 5 mm, (2) border ill defined or irregular or both (3) pigmentation irregularly distributed or associated with erythema. Naevi were classified according to the number of clinical criteria fulfilled as N3 (three criteria), N2 (two criteria) and N1 or N0 (one or no criteria). N3 and N2 naevi were counted and their location recorded.

Biopsies

The melanocytic naevus that fulfilled the greatest number of the aforesaid criteria for suspecting dysplasia in a given patient was biopsied after first being photographed. When the two clinicians chose different naevi for biopsy they consulted. Fifteen patients agreed to undergo several biopsies (at least three) in order to verify the premise that, in a given individual, if the most clinically atypical naevus is not dysplastic then the others probably are not either.

Histology

Histological examination of the slides was performed by two dermatopathologists working independently and without knowledge of the clinical data. In case of contradictory findings (three cases), the pathologists consulted. The criteria and grading of dysplasia were those described by Sagebiel *et al.*[7] In case of contention, the pathologists consulted.

Analysis of results

The correlation between the clinical and histological diagnosis was assessed. The sensitivity, specificity and the positive predictive value of the clinical examination for diagnosis of dysplastic naevus were calculated. Sensitivity corresponds to the proportion of histologically non-dysplastic naevi that were clinically designated as non-dysplastic, specificity to the proportion of histologically documented dysplastic naevi that were clinically designated as dysplastic and positive predictive value to the proportion of lesions that were histologically dysplastic out of those that were clinically designated as dysplastic. These calculations were performed on the basis of each of the two following suppositions; first that a naevus was clinically designated as dysplastic when it fulfilled three aforesaid clinical criteria (N3) and the second that a naevus was designated as dysplastic when it fullfilled at least two criteria (N2 or N3).

Data were coded for computer analysis, and clinical features in CM patients with and without dysplastic naevi were compared to determine whether subjects with dysplastic naevi constitute a separate subset.

RESULTS

Clinical findings

Of the 101 patients who finally entered the study, 4% had no melanocytic naevi at all, 17% had 1 to 10 naevi, 26% 11 to 20, 30% 21 to 50, and 23% more than 50 naevi; however only 19% had more than 5 naevi larger than 5 mm. Forty two per cent had at least one clinically atypical naevus (N2 or N3), but only 8% had N3 naevi. Five per cent had more than five clinically atypical naevi (N2) and only 1% (1 of 101) had more than five very atypical naevi (N3).

Characteristics of the most clinically atypical naevus chosen for biopsy

Biopsies were carried out on the 97 patients with melanocytic naevi. The most clinically atypical lesion was an N3 naevus in eight patients, an N2 naevus in 34, an N1 naevus in 53 and an No naevus in two patients. For most patients, the two clinicians selected the same naevus for biopsy. They disagreed in two patients with N2 naevi and in 11 patients with only N1 or No naevi, in these cases the lesion was chosen after discussion.

Histological findings

Out of the 97 most clinically atypical lesions that were biopsied, 92 were in fact naevi, but three were seborrhoeic warts and two were non-proliferative melanoses. Since biopsy revealed non-naevus lesions in five patients, it was therefore impossible to know, with the method used, whether these patients had dysplastic naevi or not. Out of the naevi which were biopsied, 17 were dysplastic, so that the estimated prevalence of dysplastic naevi in our sample was 18% (17 of 96). In eight cases dysplasia was mild and in nine it was moderate. Severe dysplasia was not observed. In the 15 patients who underwent multiple biopsies, if the most clinically atypical naevus was not dysplastic the others biopsied were not either. There was a total agreement between the two pathologists as to the histological diagnosis, but in three cases they initially disagreed about the grading of the dysplasia.

Correlation between histological findings and clinical grade

This correlation involved 97 cases since the four patients who had no melanocytic naevi were necessarily excluded. The results are shown in Table 1. The correlation between the histological diagnosis and the clinical grade was poor.

Comparison of patients with and those without dysplastic naevi

This comparison involved 96 patients, 17 with dysplastic naevi and 79 without. There were no significant differences between the two groups with respect to age, sex ratio, skin type, total number of naevi (Fig. 1a), presence and number of naevi larger than 5 mm (Fig. 1b), or the distribution of naevi on different areas of the body. In the group with dysplastic naevi there were significantly more patients with at least one N2 naevus (71% compared with 30%) and significantly more patients with at least one N3 naevus (29% compared with 4%) (Table 2). In this group there were also significantly fewer patients with naevi on the thighs (35% compared with 66%; $P<0.05$).

DISCUSSION

In several retrospective studies the prevalence of dysplastic naevi in patients with a history of malignant melanoma was assessed by seeking dysplastic naevi in histological contiguity with malignant melanoma[8–12] with a wide range of results (3 to 36%). This discrepancy could be due to differences in the phenotypes of the populations studied. However, these variations may also correspond to errors inherent in the method used. In this type of study the frequency of a histological association between dysplastic naevi and malignant melanoma may be underestimated by overlooking dysplastic naevi engulfed by cutaneous melanoma or overestimated by mistaking extensions of cutaneous melanoma for dysplastic naevi. This kind of study seeks patients in whom a dysplastic naevus may have engendered malignant melanoma and thus studies dysplastic naevi as a precursor of cutaneous melanoma.

Our method is different since we looked for dysplastic naevi other than the one that had eventually given rise to the cutaneous melanoma; thus we studied dysplastic naevi as a marker of risk rather than as a precursor. Estimated in this way, the prevalence of dysplastic naevi in our random sample of CM patients from Southern Europe was 18%. This might be an underestimate since our method may miss a solitary dysplastic naevus engulfed by cutaneous melanoma and also dysplastic naevi in patients whose most clinically atypical naevus was not dysplastic. The latter source of error is unlikely since it did not occur in the 15 cases in which multiple biopsies were performed. In a recent study[13] using similar methods (biopsy of the most

TABLE 1. Relationship between clinical grade and histological diagnosis

	Histological diagnosis (no of lesions)			
Clinical grade	Dysplastic naevi	Non-dysplastic naevi	Others	Total
No or N1	4	48	3	55
N2	8	24	2	34
N3	5	3	0	8
Total	17	75	5	97

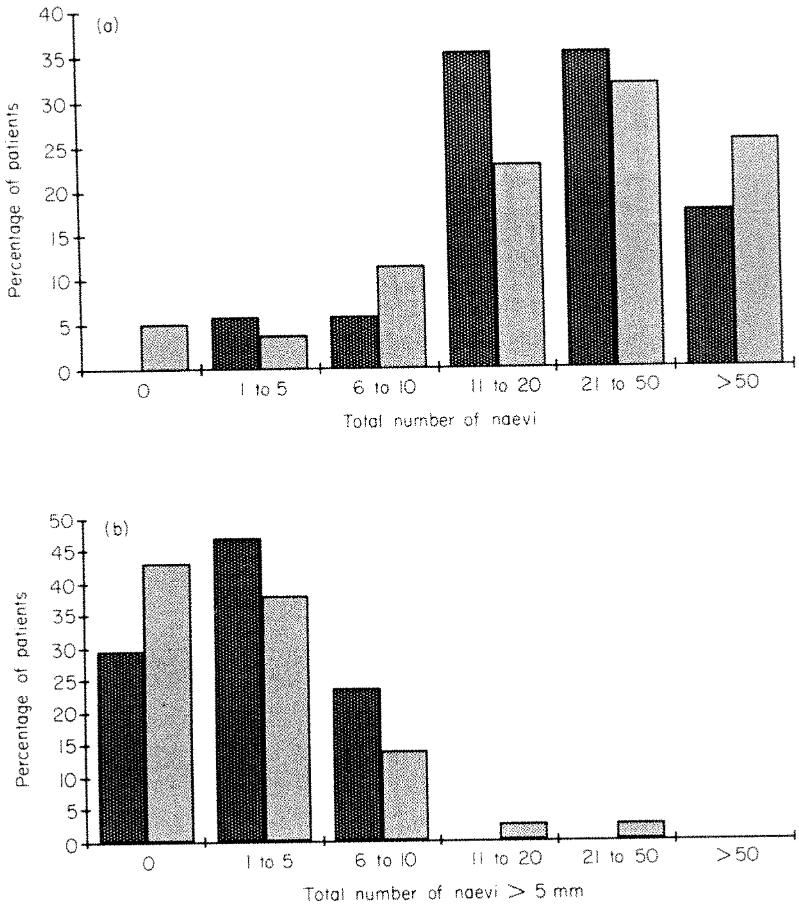

FIGURE 1. Numbers of naevi in 17 patients with dysplastic naevi (■) and 79 patients without dysplastic naevi. (▨). (a) Total numbers of naevi. (b) Naevi larger than 5 mm.

TABLE 2. Clinically atypical naevi (N2 or N3) in patients with and those without dysplastic naevi

		No of patients		
		With dysplastic naevi	Without dysplastic naevi	P*
N2 naevi	Absent	5	55	< 0·01
	Present	12	24	
N3 naevi	Absent	12	75	< 0·01
	Present	5	4	

* Chi squared test.

TABLE 3. Results of clinical diagnosis compared with results of histological diagnosis

			Histological diagnosis		
			Dysplastic	Not dysplastic	
Clinical diagnosis	Supposition 1	Dysplastic	5	3	Sensitivity = 0·29
		Not dysplastic	12	77	Specificity = 0·96
					PPV = 0·62
	Supposition 2	Dysplastic	13	29	Sensitivity = 0·76
		Not dysplastic	4	51	Specificity = 0·64
					PPV = 0·31

Supposition 1: A naevus is clinically designated as dysplastic only when it fulfils three criteria.
Supposition 2: A naevus is clinically designated as dysplastic when it fulfils two criteria.
PPV = Positive predictive value of clinical examination.

clinically atypical naevus) in 26 patients, a much higher prevalence of dysplastic naevi was reported (42%). This discrepancy may be related to differences in the populations studied.

The prevalence of clinically very atypical naevi (N3) in our sample of CM patients was 8%. In previous studies, this varied from 8 to 34%.[14–17] These differences may be attributable firstly to differences in the recognition of what is a clinically atypical naevus, secondly to variations in the mode of assessment (clinical examination or retrospective study of photographs), and thirdly to ethnic differences in the populations studied (a celtic Australian population[17] compared with a more mixed population in the present study). In any case, the prevalence of clinically atypical naevi in CM patients seem to be higher than the prevalence in the general population which has been estimated to be between 2 and 7%.[14,17,18] In our series, the patients with several atypical naevi were rare and only one patient, (i.e. 1%) could be diagnosed as having dysplastic naevus syndrome in that he had more than five N3 naevi. The fact that no other member of this patient's family had dysplastic naevi or malignant melanoma indicates that this was a sporadic case.

It must be pointed out that the prevalence of dysplastic naevi should not be taken together with that of clinically atypical naevi as has been done in certain studies. In our series, the prevalence of very atypical naevi (N3) was 8%, that of atypical naevi (N2 or N3) was 42% while that of dysplastic naevi was estimated to be 18%.

This difficulty in clinical diagnosis of dysplastic naevi is emphasized by the results in Table 3. When a naevus was clinically designated as dysplastic on the basis of three criteria (N3), the specificity of clinical examination was excellent (96%) and its positive predictive value satisfactory (62%) but its sensitivity was very poor (29%) and thus most dysplastic naevi were undetected. When a naevus was clinically designated as dysplastic on the basis of only two criteria (N2 and N3 naevi), sensitivity was satisfactory (76%), but specificity was mediocre (64%) and predictive value was poor (31%) and thus most naevi designated clinically as dysplastic were not confirmed by biopsy. Hence, we consider clinical diagnosis of dysplastic naevi to be very difficult, since, on the one hand, many dysplastic naevi are not clinically very atypical and, on the other hand, most clinically slightly atypical naevi are not dysplastic.

The same values of sensitivity and specificity may hypothetically apply to the calculation of positive predictive value of clinical examination in the general Caucasian population in which the incidence of dysplastic naevi has been estimated to be approximately 4%.[19] Because of this low prevalence, the positive predictive value would be only 23% if dysplastic naevi were diagnosed on the basis of N3 naevi and 8% if they were diagnosed on the basis of both N3 and N2 naevi. Though these extrapolations are purely speculative, they raise questions about the reliability of clinical examination for screening for dysplastic naevi in the general population. The good agreement between clinical and histological diagnosis of dysplastic naevi in some studies might be the result of the high prevalence of dysplastic naevi in the patients studied.[6]

With respect to the comparison between patients with and without dysplastic naevi, the main finding was simply that patients with dysplastic naevi more frequently had clinically atypical naevi, although in very small numbers. The parameters studied did not allow easy distinction between these two subgroups of patients. Although our survey is too small to be definite about such conclusions, it did not appear that patients with dysplastic naevi constituted a distinct subset among patients with non-familial CM. Patients with true dysplastic naevus syndrome may constitute a separate subgroup but they accounted for only 1% of patients with non-familial CM.

From a histological point of view, the criteria of melanocytic dysplasia that we followed[7] may be criticized, but appear to be reproducible. This is confirmed by the very good agreement between the two pathologists in our study.

The relatively low incidence of dysplastic naevi in patients with non-familial CM and the difficulties in clinical diagnosis of dysplastic naevi raise the question of whether clinical screening for patients with dysplastic naevi in the general population would really be a productive method of preventing non-familial CM. Prospective studies are required to answer this question. Such studies should not confuse dysplastic naevi with clinically atypical naevi, which are easier to recognize and may also be a marker for patients with a high risk of CM.[15]

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The BB-isoenzyme is a major component of creatine kinase in skin blister fluid

T.PAAVONEN, H.ARONEN, I.SAARELAINEN, H.NEITTAANMÄKI,
I.HJELM AND U.KIISTALA

Department of Pathology, University of Helsinki and Central Military Hospital, Helsinki, Finland

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SUMMARY

We measured levels of creatine kinase and its three isoenzymes in serum and blister fluid from 16 healthy volunteers. The BB-isoenzyme was found to be the predominant form in blister fluid while only the MM isoenzyme was found in serum. The levels of BB-isoenzyme in blister fluid decreased as the blisters aged. The source of BB-isoenzyme in blister fluid is most probably the damaged epidermis.

Cytoplasmic creatine kinase (CK) plays a central role in the energy metabolism of skeletal muscle, cardiac muscle and brain tissue. Of the three isoenzymes of CK, the MM component is predominant in skeletal muscle, MB in cardiac muscle and BB in brain tissue.[1,2] Mucinous epithelial cells of the intestine, and hepatocytes, contain the M polypeptide, but the benign epithelial cells of other tissues do not.[3] Low levels of MB are found in skeletal muscle, tongue, diaphragm, and esophagus, but none has been found in other tissues.[4–7]

Creatine kinase isoenzyme BB (CK-BB), first known as the brain fraction, is also the predominant isoenzyme in amniotic fluid, fetal tissues and in neonates, and belongs to the oncofetal antigens.[8–10] Furthermore, human vitreous humour and seminal fluid have been found to contain high levels of BB-isoenzyme.[11–12] The CK-BB concentration in serum was found to be elevated in marathon runners, but not in track cyclists; the rise in boxers after a fight correlated significantly with the number of blows to the head i.e. to acute brain injury.[13–15]

There have been reports of increased levels of CK-BB in malignant pleural effusions.[16] Also, the combination of a high CK-BB level in pleural fluid and a high pleural fluid: plasma CK-BB ratio has suggested that an effusion is caused by a malignant disease.[17] Recently it has been shown that especially small cell lung carcinomas are rich in CK-BB.[18] Elevated serum concentrations of CK-BB have been reported also from several other forms of cancer,[16] although its significance has recently been questioned.[19] Large amounts of the B polypeptide were found in tissue sections from many carcinomas, sarcomas and malignant fibrous histiocytomas.[3]

Skin blisters induced by a suction device, produce tissue separation at the junction between

Correspondence: Timo Paavonen, Department of Immunology, Arthur Stanley House, The Middlesex Hospital Medical School, 40–50 Tottenham Street, London W1P 9PG, U.K.

the basal lamina and the basal epidermal cells,[20] with only minor morphological signs of cellular damage, except for transient cytoplasmic vacuoles.[21,22] The clear fluid filling the blister cavity is derived from serum and from interstitial fluid from surrounding tissues. Freshly induced suction blisters usually contain neither erythrocytes nor inflammatory cells. The protein concentration is one third of the serum level, with a large predominance of albumin, thus mimicking the protein composition of extracellular interstitial fluid or lymph.[21]

Enzyme assays on suction blister fluid have been used successfully to quantitate the degree of skin irritation or damage following topical application of chemicals.[23] In the present study we used suction blisters as models for monitoring epidermal damage. Levels of creatine kinase and its isoenzymes were used as markers for cytoplasmic injury.

METHODS

Levels of creatine kinase and its isoenzymes were measured in fluid from blisters (3 to 11 mm diameter) produced by a Dermovac suction device within 2 h, using a suction pressure of 200 mm Hg on the healthy skin of 16 male subjects, aged 20 to 25 years.[20] Fluid samples were aspirated immediately after blister formation and at daily intervals from intact blisters. Serum samples were taken on day 0. Total CK was determined according to Scandinavian recommendations with an automatic analyser (LKB 8600) using reagents from Boehringer-Mannheim (Mannheim, F.R.G.).[24] After electrophoretic separation of isoenzymes on cellulose acetate, the fluorescent bands were scanned according to the manufacturer's instructions (Helena Laboratories, Beaumont, U.S.A.).

RESULTS

Of the total CK activity in blister fluid, the BB fraction comprised a mean (± SD) of 74·3 ± 11·3% as compared with 0% in serum. The mean total CK level (± SD) was 109·9 ± 70·0 U/l in fresh blister fluid and 70·0 ± 24·9 U/l in serum ($P < 0{\cdot}01$) (Table 1).

On CK isoenzyme electrophoresis a large BB faction was found in blister fluid, whereas only the MM-isoenzyme was found in serum (Fig. 1). During blister ageing only minor changes occurred in total CK activity, but there was a continuous decrease in BB concentration (Fig. 2) and a simultaneous increase in MM-isoenzyme activity (data not shown).

TABLE 1. Creatine kinase (CK) activity and proportions of CK-isoenzymes in fluid from freshly raised suction blisters and in serum

		CK U/l ($n = 16$)	Isoenzymes % ($n = 10$)		
			MM	MB	BB
Blister fluid	mean ± SD	109·9 ± 70·0	24·9 ± 10·8	0·7 ± 1·5	74·3 ± 11·7
	median	82·5			
	range	56–311			
Serum	mean ± SD	70·0 ± 24·9	98·8 ± 1·3	1·2 ± 1·3	0
	median	63			
	range	37–107			

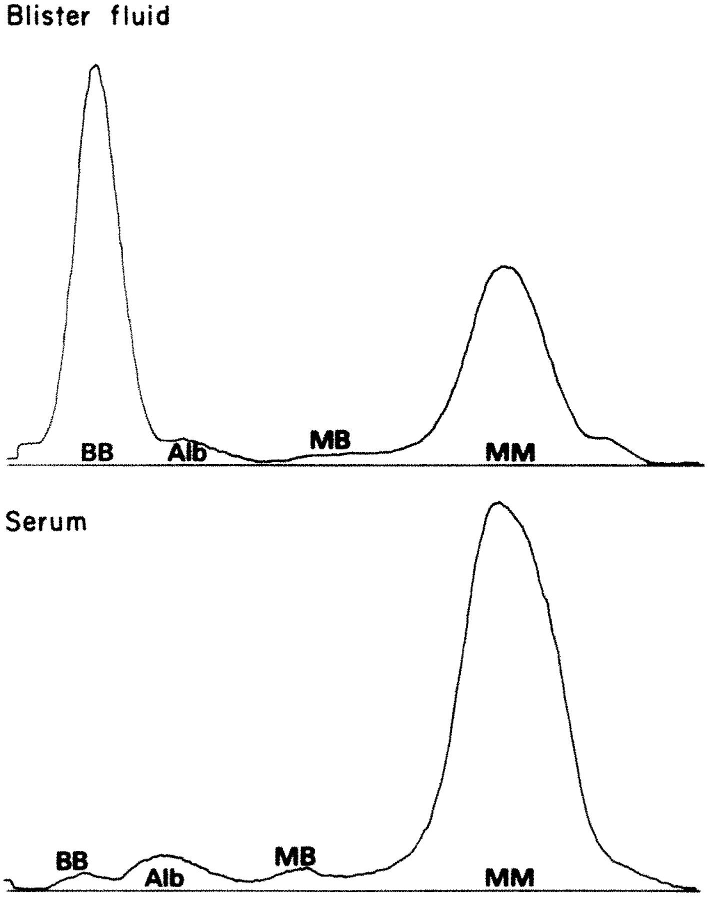

FIGURE 1. Typical tracings from creatine kinase isoenzyme electrophoresis of blister fluid and serum. Alb = albumin.

DISCUSSION

The presence of large amounts of CK-BB in human skin has not been reported previously to our knowledge, possibly because CK-BB is the least stable of the three isoenzymes of CK, having a biological half-life only 3 h.[25] The CK-MM component only has been detected in old frostbite blisters,[26] and in 2-day old burn blisters (Paavonen *et al.*, unpublished), and our present finding of a decrease in BB-isoenzyme levels with blister ageing is in accordance with these earlier findings.

It has not yet been established whether the CK-BB-isoenzyme found in blister fluid is released from the epidermis, the dermis or both. It is probably derived from mechanically injured epidermal cells, because during blister formation the cells are subjected to stretching, which enlarges intercellular spaces, puts desmosome-filament complexes under tension, and causes cytoplasmic vacuole formation.[21,22] We found that CK-BB leakage was greatest in newly formed blisters. Our preliminary results showed that separated epidermal homogenate contained six times more CK-BB than dermal homogenate (data not shown).

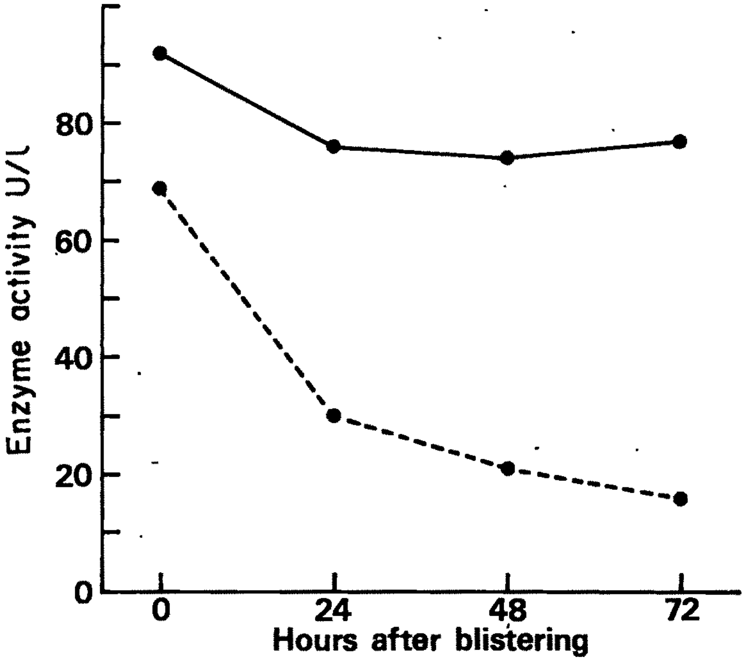

FIGURE 2. Effect of blister ageing on creatine kinase activity (———) and BB isoenzyme activity (– – – – –). Points are means for three subjects.

The distribution and function CK-BB in energy metabolism in different skin layers requires further study. Since measurement of CK and its isoenzymes is widely used in differential clinical diagnosis, it should be borne in mind that CK-BB can be released from injured skin.

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A comparative serological and molecular study of linear IgA disease and dermatitis herpetiformis

J.A.SACHS*†, J.LEONARD§, J.AWAD†, D.MCLOSKEY†,
H.FESTENSTEIN†, G.A.HITMAN‡ AND L.FRY§

*Bone & Joint Research Unit, †Department of Immunology and ‡Medical Unit, London Hospital Medical College, London and §Department of Dermatology, St Mary's Hospital, London, U.K.

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SUMMARY

The class I and class II HLA serologically defined antigens and DQα and DXα restriction fragment length polymorphism (RFLP) in 23 patients with linear IgA disease (LAD) were determined and their frequencies compared with those in a group of patients with dermatitis herpetiformis (DH) and healthy controls.

In LAD there was a significant increase in HLA-B8 and DR3 and a larger increase in the DQw1-DR2/DRw6 related DQα 6·2 kb and 6·8 kb RFLP. In DH there was a significantly increased frequency of HLA-A1, B8, DR3, and DQw2 with a concomitant increase in the DR3-DQw2 related DQα 4·6 kb RFLP. The difference in DR3 frequencies and the increased frequency of DQw1 rather than DQw2 in LAD indicates that different susceptibility genes operate in the two diseases.

Adult linear IgA disease (LAD) is a rare disorder characterized by spontaneous sub-epidermal blisters and the presence in the uninvolved skin of a linear deposit of IgA along the dermo-epidermal junction.[1] Although 24% of patients with LAD have been shown to have an associated enteropathy, there is no evidence that the rash is gluten sensitive.[2] Previous studies of class I antigens showed an incidence of HLA-B8 of about 55% which was significantly higher than the frequency of 30% in controls.[1,3] There have been no reports to our knowledge of the incidence of class II antigens in LAD.

Dermatitis herpetiformis (DH) is characterized by the presence of spontaneous sub-epidermal blisters and IgA deposits in the papillary dermis of uninvolved skin.[4] There is an associated gluten sensitive enteropathy and both the gut and the skin lesions respond to a strict

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Correspondence: Dr J.A.Sachs, Department of Immunology, London Hospital Medical College, Turner Street, London E1 2AD, U.K.

gluten free diet.[5] A strong HLA association has been found with HLA-A1, B8 and DR3—approximately 90% of patients have B8 or DR3 or both.[6,7]

We have recently measured the frequency of the serologically detectable HLA class I (A, B and C) and class II (DR and DQ) antigens[8] and class IIα chain restriction fragment length polymorphism (RFLP)[9] in British Caucasoid patients with DH and compared their frequencies with those in a control group.

In the present study we have compared the frequencies of HLA class I and II antigens, and RFLPs derived from DQα probes in 23 European Caucasoid LAD patients, with those in DH patients and healthy controls.

METHODS

Subjects

Twenty three Caucasoid patients (11 men and 12 women) with a diagnosis of LAD were studied. The results were compared with the data accumulated from a panel of 83 patients with DH[9] and a panel of up to 309 healthy controls.[10] The criteria for inclusion in the study for the two disease groups were as follows: LAD was characterized by the presence of spontaneous sub-epidermal blisters and a homogeneous linear deposit of IgA along the dermo-epidermal junction of uninvolved skin. Four of the LAD patients had linear IgG deposits also. All patients studied were over the age of 12 years at the onset of their disease, thus distinguishing this group from benign chronic bullous dermatosis of childhood.[1] DH was characterized by the presence of spontaneous sub-epidermal blisters and deposits of IgA in the papillary dermis of uninvolved skin.

Serological typing for class I and class II antigens

Twenty three patients with LAD and 83 with DH were typed for class I and class II antigens using previously described techniques.[8] Of the 309 controls typed for class I antigens, 286 were also typed for DR and DQ, of which 96 were used in RFLP studies.

DNA analysis

DNA was extracted from leukocytes obtained from whole blood samples anticoagulated with EDTA and stored at −20°C. The DNA was then digested with the enzyme Taq 1 and analysed by standard techniques as described previously.[9] Seventeen patients were studied and the results compared with those from 63 patients with DH and 96 healthy controls. The DQα probes detected Taq 1 polymorphisms of the DQα (6·8, 6·2, 5·5, 4·6 and 2·6 kb) and DXα (2·1 and 1·9 kb) genes. An example of the autoradiograph produced from 7 individuals is shown in Figure 1.

Statistical analysis

Serological and RFLP data were analysed for differences in allelic or genotypic frequencies by Chi square tests using 2 × 2, 3 × 2 or 5 × 2 contingency tables.

Jejunal biopsies

Jejunal biopsies were performed on 21 of the patients with LAD using a Crosby capsule. The biopsies were examined with a dissecting microscope for morphological abnormalities and also histologically for the presence of increased lymphocytic infiltration of the epithelium.[11]

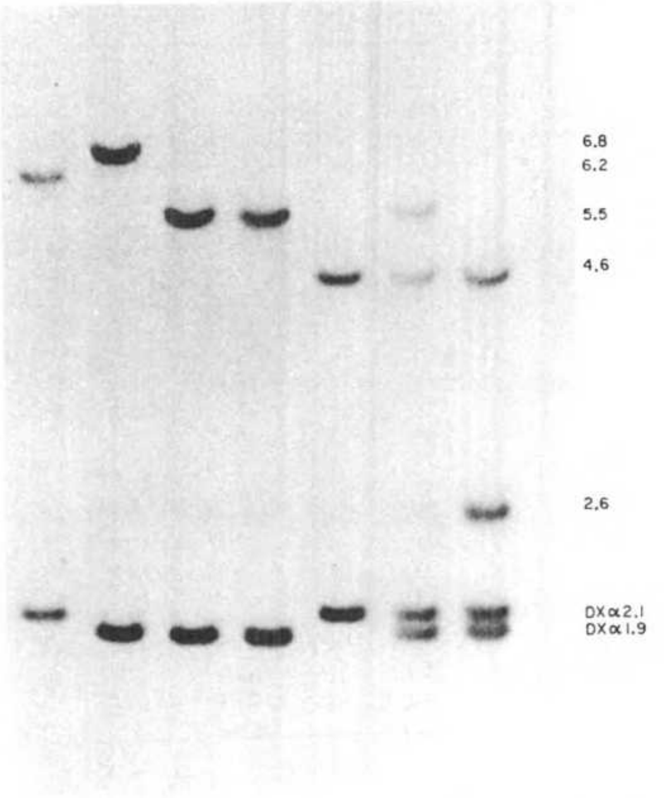

FIGURE 1. Autoradiogram of DQα region RFLP. The numbers indicate the size in kb of the different fragments. DNA from seven individuals was digested with the restriction enzyme Taq 1 and hybridized with the DQα genomic probe using the Southern blot technique. Note that the DQα probe also hybridizes with DXα 2·1 and 1·9 kb fragments.

RESULTS

The HLA-A, B, C, DR and DQ antigenic and DRα and DXα RFLP profile of each LAD patient is given in Table 1. The frequency of the different DR and DQw serologically defined antigens in LAD, DH and the control groups are compared in Table 2. In LAD, the incidence of both B8 and DR3 was higher than in the controls (55% compared with 21%, $P < 0{\cdot}01$ and 53% compared with 26%, $P < 0{\cdot}05$, respectively); while in DH the incidence of both antigens was very much higher than in the controls (81% compared with 21%, $P < 0{\cdot}001$, and 88% compared with 26%, $P < 0{\cdot}001$, respectively). The incidence of DRw6 was significantly higher in LAD (32%) than in DH (8%) ($P < 0{\cdot}01$). The frequency of DQw1 in LAD (79%) was not significantly higher than in the controls (64%), but was significantly higher than that in patients with DH (51%) ($P < 0{\cdot}05$). The frequency of DQw2 in LAD (47%) was not significantly different from the controls (40%), but was significantly less than in patients with DH (94%, $P < 0{\cdot}001$).

The frequency of the DR3-DQw2 associated 4·6 kb RFLP was not significantly different between the LAD group (59%) and the controls (44%), but was considerably higher in DH

TABLE 1. Results in patients with linear IgA disease

Patient no.	Sex	Skin biopsy*	Jejunal biopsy†	HLA				DQα kb	DXα kb
				A	B	DR	DQw		
1	M	IgA	1	1/3	8/39	NT	NT	6·2/6·2	1·9/1·9
2	M	IgA	1	3/28	7/8	1/2	1	NT	NT
3	F	IgA, IgM	1	1/3	27/57	10/13	1	6·8/6·2	2·1/2·1
4	F	IgA, IgM	1	2/3	50/60	NT	NT	6·2	2·1/2·1
5	F	IgA	1	2/31	7/44	9/13	1/3	NT	2·1/1·9
6	M	IgA	1	19/28	12/17	2/4	1/3	NT	NT
7	M	IgA	1	1/3	7/8	2/4	1/3	6·2/5·5	2·1/1·9
8	M	IgA, IgM	1	2/11	18/38	2/6	1	6·8/6·2	1·9/1·9
9	M	IgA	1	1/33	8/65	3/11	2/3	4·6	2·1/1·9
10	M	IgA	1	3/25	18	2/3	1/2	6·2/4·6	2·1/1·9
11	M	IgA	3	1/3	8/39	2/3	1/2	6·2/4·6	2·1/2·1
12	M	IgA	1	1/3	7/8	2/3	1/2	6·2/4·6	2·1/2·1
13	F	IgA	1	1/2	47/55	3/13	1/2	6·8/4·6	2·1/1·9
14	M	IgA	1	2/24	8/18	3/13	1/2	4·6/2·6	2·1/1·9
15	F	IgA	3	2/25	8/51	3/13	1/2	6·8/4·6	2·1/1·9
16	F	IgA	3	2	8/44	3/7	2	NT	NT
17	F	IgA	1	2/11	18/35	4	3	NT	NT
18	M	IgA	2	NT	NT	NT	NT	6·2/4·6	2·1/2·1
19	F	IgA, IgM	NT	2/29	18/27	NT	NT	6·2/4·6	2·1/2·1
20	F	IgA, IgG	NT	1/11	7/8	4/13	1/3	6·8/5·5	2·1/1·4
21	F	IgA, IgG	1	1/2	7/8	2/3	1/2	6·2/4·6	2·1/2·1
22	M	IgA, IgG, IgM	1	1/3	7/8	3/9	2/3	5·5/4·6	2·1/1·9
23	F	IgA, IgG, IgM	1	3/11	12/w40	5	3	NT	NT

* Immunofluorescence.

† 1 = Normal; 2 = Normal villi but increased intra-epithelial lymphocyte count (IELC); 3 = Convoluted mucosa, and increased IELC.

NT = not tested.

patients (90%, $P < 0{\cdot}001$). In LAD the frequencies of the DQw1 associated 6·8 kb and 6·2 kb RFLPs were significantly higher than in the control and the DH groups; 6·8 kb or 6·2 kb RFLP or both were found in 14 of 17 (82%) LAD patients compared with 23 of 63 (43%) DH patients ($P < 0{\cdot}01$) and 39 of 91 (43%) controls ($P < 0{\cdot}01$). The DXα polymorphism (2·1/2·1 kb, 2·1/1·9 kb, 1·9/1·9 kb) showed a significantly lower frequency of the 1·9/1·9 kb pattern in both DH and LAD (Table 3). This reflects the association of the 2·1 kb RFLP with DR3 and the non-DR3 association of the 1·9 kb fragment.

The jejunal biopsy results in 21 of 23 patients are listed in Table 1. Four of the 21 patients with LAD who had had a jejunal biopsy taken were found to be abnormal; one patient had a morphological abnormality and the other three patients had a raised intra-epithelial lymphocyte count (IELC). There was no obvious relationship between the presence of an enteropathy and any HLA profile.

TABLE 2. Distribution of class I and class II antigens in patients with linear IgA disease, DH and normal controls

Antigen	Linear IgA %	DH %	Controls %
	($n=22$)	($n=83$)	($n=309$)
A1	45	63**	32
B8	55**	81***	21
	($n=19$)	($n=83$)	($n=286$)
DR1	5	7	19
DR2	42	35	31
DR3	53§*	88***	26
DR4	21	20	35
DR5 (DR11 and 12)	11	2	15
DR6 (DR13 and 14)	32‡	8	19
DR7	5	18	31
DR2/3	17*	35***	5
DR3/7	4	14	6
DR3 alone	0	8	4
DQw1	79†	51	64
DQw2	47§	94***	40
DQw3	38	27	55

Compared with controls * $P<0{\cdot}05$, ** $P<0{\cdot}01$, *** $P<0{\cdot}001$.

Compared with DH patients † $P<0{\cdot}05$, ‡ $P<0{\cdot}01$, § $P<0{\cdot}001$.

TABLE 3. Distribution of DQα and DXα RFLP after digestion with Taq I enzyme

DQα	6·8 kb %	6·2 kb %	5·5 kb %	4·6 kb %	2·6 kb %
Linear IgA disease ($n=17$)	35*†	53*†	18†*	59§	24
DH ($n=63$)	13	25	46	90***	11
Controls ($n=91$)	11	29	66	44	25

DXα	2·1/2·1 %	2·1/1·9 %	1·9/1·9 %
Linear IgA disease ($n=18$)	29	59	12*
DH ($n=65$)	49	45	6***
Controls ($n=96$)	18	46	35

Compared with controls * $P<0{\cdot}05$, ** $P<0{\cdot}01$, *** $P<0{\cdot}001$.
Compared with DH patients † $P<0{\cdot}05$, ‡ $P<0{\cdot}01$, § $P<0{\cdot}001$.

DISCUSSION

The present study has demonstrated that there was a significantly increased frequency of DR3 in the LAD patients (53%) compared with controls (26%) but the increase did not approach that in DH (88%). This could reflect the relatively small number of LAD patients studied or it may correspond to other HLA-DR3 associated disorders which have a similar 'midway' DR3 frequency of between 50–60%, such as systemic lupus erythematosus[12] and insulin dependent diabetes mellitus.[13]

The combined serological and RFLP data focus attention on the DQ region as being of relevance in both groups of patients. However, DQw1 is associated with DRw6 in LAD and not DR2 which was found in DH patients.[8] Furthermore, the distribution of the DQα 6·2 kb and 6·8 kb fragments in the LAD patients was distinctly different from both the DH patients and healthy controls. These findings point to significant immunogenetic differences between DH and LAD. Taken together with other evidence that has been presented over the past 5 years[2,14,15] the results of the present study point to the conclusion that DH and LAD represent distinct disease entities.

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The structure of fucosylated blood group substances in fetal rat skin. The combined use of monoclonal antibodies and glycosyl hydrolases

SUSAN E.KELLY AND S.FLEMING*

Department of Dermatology, Royal Berkshire Hospital, Reading and
*Department of Pathology, University of Southampton, Southampton, U.K.

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SUMMARY

Cell surface carbohydrates are thought to be important during embryonic development. We have studied a group of defined cell surface carbohydrates during the differentiation of fetal rat skin by the use of immunocytochemical techniques. We used monoclonal antibodies to the Le X and human blood group H type II haptens. The structure of the molecules carrying these haptens has been inferred by the sensitivity of the immune reactions to digestion by glycosyl hydrolases.

The primitive ectoderm expressed the Le X hapten. The differentiating epidermis carries the H type II antigen, but this was only detected following cleavage of a terminally modified portion of the carbohydrate containing linear poly-N-acetyl lactosamine chains. These carbohydrates on epidermal cells are carried N linked to glycoproteins.

The cell surface expression of fucosylated derivatives of poly-N-acetyl lactosamine has been shown to be important during mammalian development.[1] These glycoconjugates have been shown to be expressed in a characteristic distribution[2,3] and to be involved in cell interactions at the morula stage and during gastrulation of the pre-implantation mammalian embryo.[4] Related molecules are expressed on the cell surface of inducer tissues during organogenetic interactions,[5] and on the surface of some cells during differentiation of fetal tissues.[6]

Demonstration of these molecules has been achieved by utilizing the specificity of monoclonal antibodies.[7] The monoclonal antibodies used in these investigations have been specific for the terminal oligosaccharide epitopes which are carried on larger carbohydrate side chains of glycolipids or glycoproteins.[8] It is now possible to analyse parts of the structure of the remainder of the carbohydrate side chains by the use of glycosyl hydrolases.[3] These are enzymes which hydrolyse linkages of a specific configuration between defined saccharrides. Thus, by testing the sensitivity of monoclonal antibody binding in immunocytochemical experiments to pre-

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Correspondence: Dr S.E.Kelly, Department of Dermatology, Royal Berkshire Hospital, Reading, Berkshire RG1 5AN, U.K.

digestion by specific glycosyl hydrolases, the structure of the carbohydrate side chain carrying the epitopes may be inferred.

We have studied the distribution of fucosylated derivatives of the type II blood group substance during the development and differentiation of fetal rat skin using monoclonal antibodies and the immunoperoxidase technique, and we have further analysed the structure of these important glycoconjugates using glycosyl hydrolases.

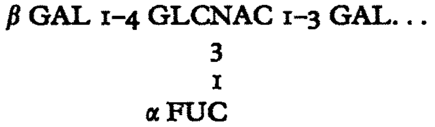
METHODS

Tissues

Adult Sprague-Dawley rats were mated overnight and the presence of a vaginal plug on morning examination taken as evidence of successful mating. Animals were killed on gestational days 11, 13, 15, 17, 19 and at term. The fetuses were dissected and fixed in Bouin's fixative. Previous work has shown this to be the optimal fixative for demonstration of the appropriate carbohydrate.[9] The tissue was processed to paraffin wax and a 4 μm section cut and stained with haematoxylin and eosin. Multiple adjacent sections were cut and stained by immunocytochemical methods.

Antibodies

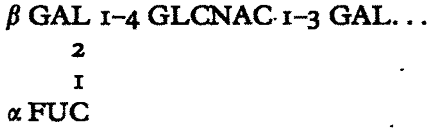
The murine monoclonal antibodies AGF 4·48 and Leu M1 are both reactive against the Le X hapten. This antigenic determinant has the structure:



$$\beta\,\mathrm{GAL}\ 1\text{–}4\ \underset{\substack{3\\1\\\alpha\,\mathrm{FUC}}}{\mathrm{GLCNAC}}\ 1\text{–}3\ \mathrm{GAL}\ldots$$

This oligosaccharide is also responsible for the reactivity of the antibody SSEA-1 and the human leukocyte marker CD15. AGF4·48 shows CD15 like immunoreactivity in immunocytochemical testing[10] and its specificity for the above oligosaccharide has been confirmed by competitive inhibition of binding using synthetic oligosacchrides.[10] Leu M1 is a commercially available CD15 monoclonal antibody (Dako).

A commercially available monoclonal antibody against the human blood group substance H on a type II precursor chain was also used. The epitope recognized by this antibody has the structure:



$$\underset{\substack{2\\1\\\alpha\,\mathrm{FUC}}}{\beta\,\mathrm{GAL}}\ 1\text{–}4\ \mathrm{GLCNAC}\ 1\text{–}3\ \mathrm{GAL}\ldots$$

All three of these antibodies are murine IgM molecules.

Glycosyl hydrolases

Several different glycosyl hydrolases were used. Sections were incubated with these enzymes before incubation with the primary antibodies in the immunocytochemical technique. Neuraminidase (acylneuraminyl hydrolase EC 3.2.1.18) from *Clostridium perfringens* removes terminal neuraminic acid from carbohydrate chains. Tissue sections were incubated with neuraminidase for 2 h at room temperature at pH 4·6 in phosphate buffer.

β-Galactosidase (β-D-galactoside galactohydrolase EC 3.2.1.23) is an exoglycosidase which digests terminal galactoside residues from carbohydrate chains. The enzyme used was synthesised from a recombinant *E.coli* strain. Sections were incubated with this enzyme at an activity of 1 U/ml at pH 7·0 in phosphate buffer. α-Fucosidase (α-L-fucoside fucohydrolase EC 3.2.1.51) from beef kidney removes terminal α-fucoside residues from carbohydrate chains. Sections were incubated with this enzyme at 1 U/ml at pH 5·0 in ammonium sulphate buffer.

The structure of the poly-N-acetyl lactosamine carbohydrate backbone of these molecules can be inferred from the sensitivity to digestion by endo-β-galactosidase.[11] Endo-β-galactosidase hydrolyses GAL 1–4 GLC NAC linkages when they occur within unbranched chains of this disaccharide unit, but it will not digest branched chains of the same carbohydrates. It, therefore, distinguishes between the human blood group antigen i molecule which is enzyme sensitive and blood group antigen I molecule which is enzyme resistant. Endo-β-galactosidase (EC 3.2.1.103) purified from *Bacterioides fragilis* by the method of Scudder *et al.*,[11] was incubated with tissue sections at an activity of 0·1 U/ml at pH 5·8 in veronal buffer.

N-linked oligosaccharides are sensitive to digestion by glycopeptidase-F. This enzyme cleaves high mannose, hybrid and complex glycans from glycoproteins between the Asn of the peptide and the GLCNAC of the carbohydrate.[12] The glycopeptidase-F (EC 3.2.2.18) used in these experiments was purified from *Flavobacterium meningosepticum* and was used at an activity of 1 U/ml at pH 7·5 in phosphate buffer.

Endoglycosidase F from *Flavobacterium meningosepticum* (endo-β-N-acetylglucosaminidase F, EC 3.2.1.96) cleaves high mannose, hybrid or complex saccharides from peptide chains. This enzyme was used at an activity of 1 U/ml at pH 5·0 in phosphate buffer. With the exception of neuraminidase all other incubations were performed overnight (16 h) at 37°C.

The cleavage sites of the glycosyl hydrolases on N-linked oligosaccharides are shown in Figure 1.

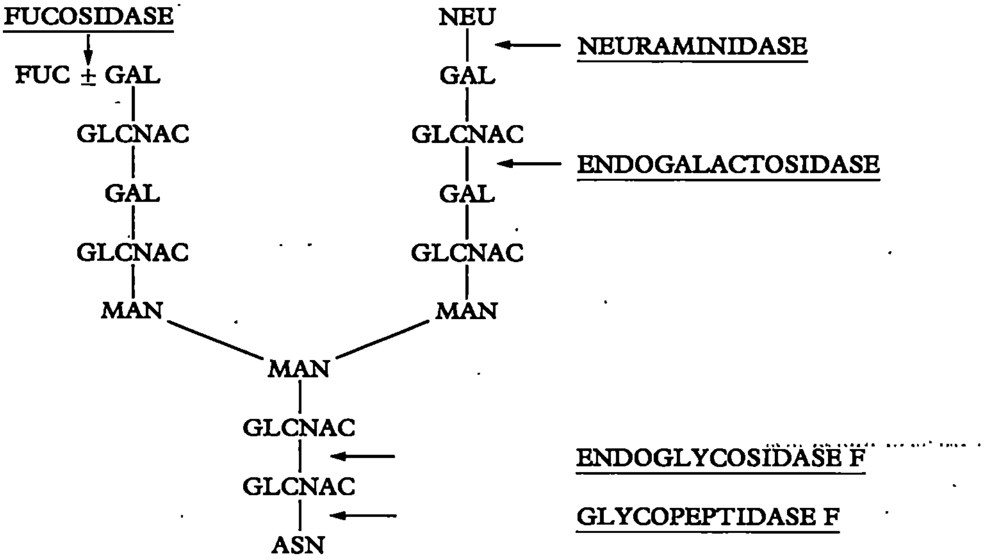


FIGURE 1. The structure of N-linked biantennary oligosaccharides indicating the sites of cleavage of the glycosyl hydrolases used in the study.

All of the above glycosyl hydrolases were obtained from Boehringer-Mannheim (Mannheim, F.R.G.).

Immunocytochemistry

Antibody binding to tissue sections was visualized using the avidin-biotin peroxidase complex method.[13] The reagents used were obtained as an ABC immunocytochemistry kit from Vector Laboratories, (Burlingame, U.S.A.).

Control experiments

Buffer controls were performed by incubating sections with the buffer solutions which were used to perform the digestions by the glycosyl hydrolases.

The specificity and sensitivity of the enzyme and immunocytochemical reactions were confirmed by performing the simultaneous experiments on human fetal kidney, a tissue in which the structure of fucosylated poly-N-acetyl lactosamine derivatives has been previously demonstrated using these techniques.[9]

Standard immunocytochemistry controls were performed. The antigenic structure demonstrated by the binding of the monoclonal antibodies was confirmed by pre-digestion by the exoglycosidases β-galactosidase and α-fucosidase. These enzymes cleave from the carbohydrate chain the terminal galactoside and fucoside residues which confer the specificity of the Le X and H type II epitopes. In the immunocytochemical experiments binding of the monoclonal antibodies was consistently sensitive to predigestion of the tissue sections by these exoglycosidases.

RESULTS

Examination of haematoxylin and eosin stained sections confirmed the gestational ages of the festuses and that development was entirely normal.

Distribution of Le X hapten in fetal rat skin

In the 11-day gestation rat fetuses strong immunoreactivity for Le X was seen on the external surface of the ectoderm (Fig. 2). Staining was particularly pronounced on the thickened ectoderm over the head region. Staining was seen at this site in day 13 fetal skin but was lost with further development of the periderm and epidermis.

The ectodermal staining for Le X was lost following predigestion of the tissue sections using the endoglycosidases glycopeptidase F, endoglycosidase F and endo-β-galactosidase.

Distribution of H type II substance in fetal rat skin

No immunoreactivity for H type II substance was seen in the ectoderm of day 11 nor the periderm of day 13 fetal rats. Reactivity was not exposed by treating the sections with glycosyl hydrolases.

In fetal rat skin at the later stages of development, that is days 15, 17 and 19, H type II substance was not detected on untreated sections. On sections which had been pre-treated with endo-β-galactosidase, however, intense staining for this hapten was seen in the fetal skin. Staining was seen in the superficial layers of the developing epidermis, but not in the basal cells (Fig. 3). This immunoreactivity was sensitive to predigestion of the tissue sections by glycopeptidase-F and endoglycosidase-F.

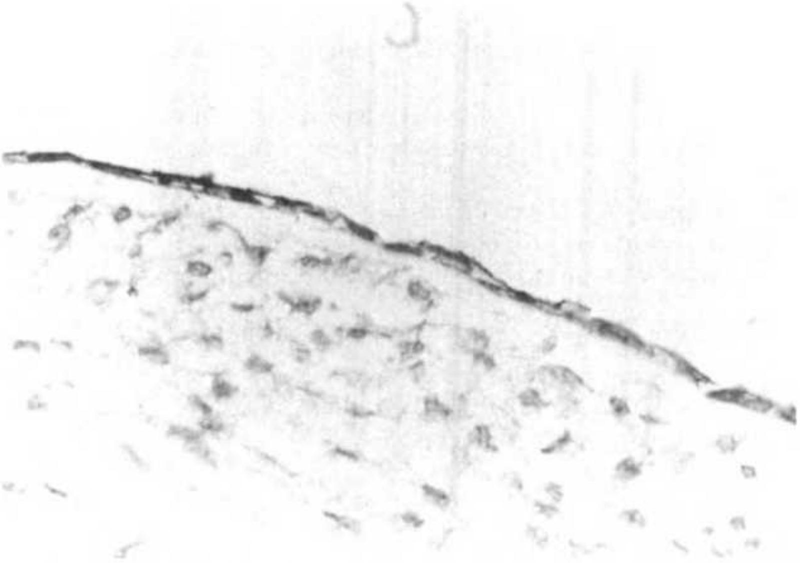


FIGURE 2. The ectodermal layer of a day 11 rat fetus showing binding of Leu M1 to the single layer of epithelial cells. Immunoperoxidase.

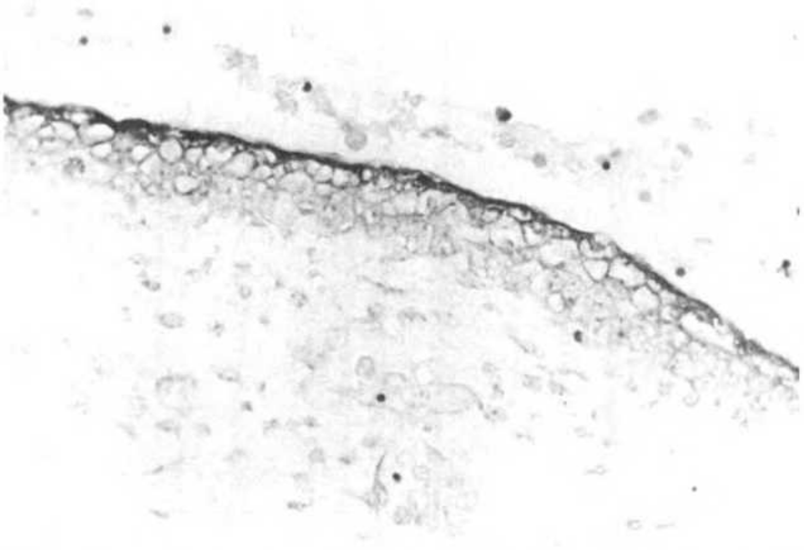


FIGURE 3. Following digestion of tissue sections by endo-β-galactosidase, reactivity for H type II substance can be seen on the superficial cells, but not the basal cells of the developing rat epidermis. Immunoperoxidase.

In developing vibrissa follicles, intense staining for H type II was seen following digestion of the tissue by endo-β-galactosidase. The staining was seen in the cells of the hair bud at the early stages of development and later in the superficial keratinocytes of the hair bulb, but not in the basal cells nor hair papilla (Fig. 4).

The statified squamous epithelium of the developing buccal mucosa did not express the H type II substance even after endo-β-galactosidase treatment (Fig. 5).

Distribution of Le X hapten and H type II substance in adult rat skin

In untreated sections of adult rat skin immunoreactivity for neither of these determinants was seen. The determinants were not exposed by pretreatment with glycosyl hydrolases.

Control experiments

The detection of the appropriate antigens by the monoclonal antibodies was unaffected by preincubation of tissue sections in the buffer solutions in which the enzymes were diluted. Immunocytochemistry control sections were negative.

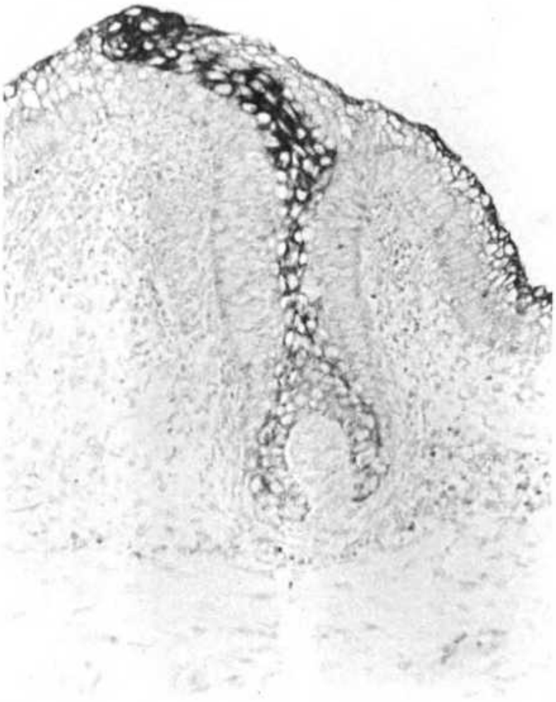


FIGURE 4. Hair bulb showing the expression of H type II substance after pre-digestion by endo-β-galactosidase. Immunoperoxidase.

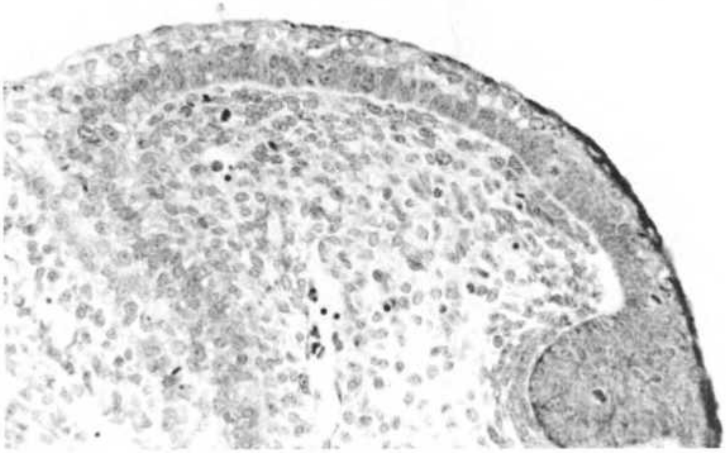


FIGURE 5. Mucocutaneous junction in which the skin and hair bud show expression of H type II substance, but buccal mucosa and dental lamina do not. Immunoperoxidase following endo-β-galactosidase.

DISCUSSION

In this study we have analysed the structure of the carbohydrates which are responsible for antigenicity related to the human type II blood group substances in the fetal rat skin. These molecules are fucosylated derivatives of N-acetyl lactosamine.[1]

The pattern of expression of these molecules was found to change during development of the fetal skin. Similar changes have been noted during the development of other tissues in both human[5] and rodent fetuses.[2,3] Changes related to those which we have described in fetal rat skin have, recently, also been noted during the development of fetal human skin.[14]

The primitive ectoderm of the rat expressed the Le X hapten. This epitope was carried on a molecule which showed sensitivity to digestion by endo-β-galactosidase. The specificity of this enzyme for linear forms of poly-N-acetyl lactosamine[11] suggests that the Le X epitope is the terminal oligosaccharide of such a molecule in the embryonic rat ectoderm. Rastan *et al.*[3] have previously shown that the same glycoconjugate is expressed on the ectoderm of the murine embryo at an equivalent developmental stage. Immunoreactivity for Le X is subsequently lost from this site in the rat as in the mouse.[3]

During the development of the epidermis a different form of fucosylated N-acetyl lactosamine was found. The fetal rat skin at this later developmental stage contained molecules which bound a monoclonal antibody to human blood H type II. Several groups have described binding of the lectin from *Ulex europeus* (UEA) to parts of the fetal rat skin.[15] UEA binding is found in the spinous and lower granular layers of the fetal rat epidermis. The UEA lectin binding site has been shown to include the trisaccharide which confers H type II immunoreactivity.[16] It seems likely, therefore, that these two separate techniques of immunocytochemistry and lectin histochemistry are recognizing the same molecule in the fetal rat epidermis.

Brown *et al.*[15] in their lectin histochemistry study showed that as UEA reactivity was lost during the terminal differentiation of keratinocytes, reactivity for other lectins was acquired. Specifically lectins recognizing galactose and glucosamine may bind to terminally differentiated keratinocytes.

In the present study we have found that pre-treatment of tissue sections with endo-β-galactosidase exposed reactivity for H type II epitopes on the surface of terminally differentiated keratinocytes. This suggests that the H type II epitope may be masked on differentiated keratinocytes by elongation of a linear non-fucosylated poly N-acetyl lactosamine chain resulting in a molecule with the structure outlined in Figure 6. Our interpretation of these data and of the lectin histochemistry experiments is that, rather than the degradation of the fucosylated molecules suggested by Brown *et al.*,[15] there is terminal modification of the oligosaccharide chain. At present neither we nor Brown *et al.* can exclude the possibility that the H type II reactivity and the masking non-fucosylated polylactosamine chains are on different molecules.

Glycoconjugates of the polylactosamine group are frequently carried as N-linked saccharides on surface membrane glycoproteins.[8] N-linked oligosaccharides can exist in several forms. Fleming[9] has previously shown that these structures can be analysed by immunocytochemical methods using certain endoglycosidases. We have further analysed the nature of the glycoconjugates of fetal rat skin by the use of the enzymes glycopeptidase F and endoglycosidase F. These enzymes cleave specific parts of N-linked oligosaccharides.[12,17] Both forms of the fucosylated N-acetyl lactosamine epitope on the fetal rat skin were sensitive to digestion by these enzymes. Glycopeptidase F hydrolyses the bond between glucosamine, the first saccharide in the carbohydrate side chain, and the asparagine residue on the peptide molecule.[12] Endoglycosidase F cleaves high mannose, non-bisected biantennary hybrid or complex structures.[17] The results of these enzyme digestion experiments suggest that the fucosylated N-acetyl lactosamine molecules on fetal rat epidermal cells have the structure outlined in Figure 6.

The expression of fucosylated derivatives of poly-N-acetyl lactosamine must undergo

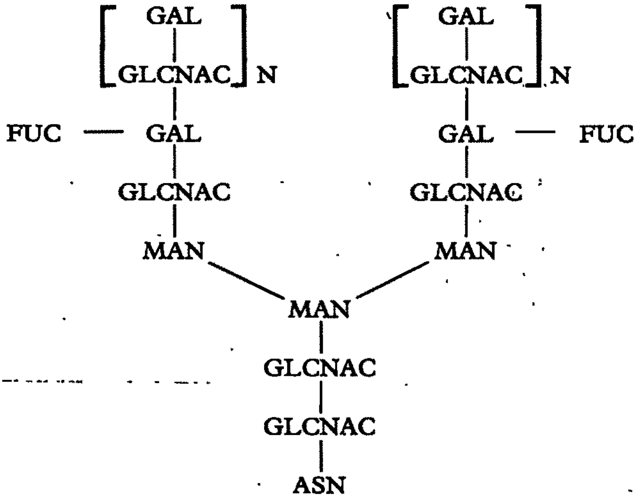


FIGURE 6. Proposed structure of the fucosylated N-acetyl lactosamine oligosaccharide on the differentiating rat epidermis. N represents linear extension of the poly N-acetyl lactosamine chain.

regulated changes during epidermal differentiation. The significance of such precise control of these molecules is not clear. Carbohydrates of this type are known to be differentiation antigens in normal and neoplastic development and also to be involved in receptor systems.[1] These molecules have been identified on the epidermal growth factor receptor on A 431 cells and changes in the carbohydrate components regulate the activation of the receptor.[1] Therefore, the changes we have observed during the differentiation of fetal rat skin may be related to activation or regulation of epidermal receptor molecules.

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Immune responses to herpes simplex virus in patients with facial herpes simplex and those with eczema herpeticum

J.P.VESTEY, S.E.M.HOWIE,* M.NORVAL,* J.P.MAINGAY* AND W.A.NEILL*

Departments of Dermatology and *Bacteriology, University of Edinburgh Medical School, Teviot Place,
Edinburgh EH8 9AG, U.K.

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SUMMARY

The immune response to herpes simplex virus (HSV) was studied in 59 patients with primary and recrudescent facial HSV infections. The patients included nine with atopic eczema, seven of whom had eczema herpeticum (EH). All patients had antibodies to HSV (measured by ELISA) and all but three had HSV-specific cell mediated immunity (CMI) (measured by *in vitro* lymphoproliferation). Thirteen control subjects were negative for both tests. All three patients with absent CMI to HSV had suffered from severe EH and had depressed CMI to HSV for several months following an attack. In two of these EH patients, a positive CMI response was produced by *in vitro* removal of CD8 +ve T lymphocytes from peripheral blood mononuclear cells using a panning technique. Thus the absence of CMI to HSV in these patients was due to suppressor cell function rather than a lack of specifically responsive cells. The other four EH patients with normal CMI to HSV had suffered less severe EH, but no association between the absence of CMI to HSV and serum IgE level or activity of the eczema was apparent in the atopic patients. No specific anti-HSV IgE antibody was detectable.

Following a primary infection with herpes simplex virus (HSV), nearly all patients are thought to develop lifelong latent infections. A significant proportion of these patients may then undergo reactivation and secretion of virus at intervals.[1-3] Clinically apparent recrudescent lesions ('cold sores' or 'fever blisters') also occur in up to 50% of infected patients, often in association with well known triggering factors such as sun exposure, other infections, hormonal changes, or local trauma.

HSV specific antibody, delayed type hypersensitivity, *in vitro* lymphoproliferative responses, cytotoxic T cell production, antibody dependent cytotoxicity and T cell production of lymphokines and interferon (α and γ) in response to HSV antigenic stimulation have all been

Correspondence to: Dr J.P.Vestey.

detected in patients following primary HSV infection.[1-4] Several of these responses can be elicited for many years following recovery from HSV infection even if recrudescence does not occur.

Work with animal models[5] suggests that antibody may play a role in preventing the spread of HSV infection to the nervous system, but cell mediated immunity (CMI) appears to be most important in recovery from acute HSV infections.[1-4,6] Patients with impaired CMI to HSV because of treatment with immunosuppressive drugs for transplantation or cancer chemotherapy suffer from more frequent and severe HSV infections than healthy individuals, despite normal or near normal antibody levels.[4,7]

Several authors have detected a slight depression of lymphoproliferative responses *in vitro* to HSV in patients during recrudescences.[8-10] Depressed NK cell activity and production of lymphokines and interferon by lymphocytes from such patients have also been noted.[6,11-14] These reductions in CMI responses appear to improve during convalescence, rise to a maximum level of activity 3 to 8 weeks after recrudescence and then decline slowly to a low level. However, they remain significantly higher than results obtained with seronegative controls. The temporary impairment of CMI to HSV during recrudescence has been associated with a transient relative increase in circulating CD8 +ve cytotoxic/suppressor T cells[6] and virus specific production of suppressor factors *in vitro* by CD8 +ve T cells[13,15] in HSV type 2 (HSV-2) infections.

On the other hand, such a picture of impaired CMI during recrudescence has not been found by all workers. Several studies describe lymphoproliferation, lymphokine production and cell mediated virus specific cytotoxicity as being greater during recrudescence than convalescence followed by a slow decline to background levels until the next attack;[4-11] others suggest that the lowest level of NK cell activity occurs just before a recrudescence with a rapid rise during the attack followed by a gradual decline to a background positive level.[14]

Initially the present study was undertaken to clarify the relationship between CMI and antibody responses to HSV in healthy patients with different frequencies of recrudescences and different triggering factors precipitating recrudescences. Within the patient group there was a small number with eczema herpeticum (EH). Some of these showed no CMI to HSV, which led us to study the role of suppressor cell function in the control of HSV infection.

METHODS

Patients

Fifty-nine patients (18 male, 41 female; mean age 33 years, range 9–60 years) with facial HSV but who were otherwise well, and 13 healthy controls (five male, eight female; mean age 32 years, range 22–45 years) were studied. Patients were either referred to the Dermatology Department at the Edinburgh Royal Infirmary because of their HSV or were recruited from members of staff; control subjects were staff members with no history of a previous HSV infection.

Blood samples were taken for measurement of HSV antibodies by ELISA and of CMI to HSV by *in vitro* lymphoproliferation, and for a full blood count, biochemical profile and measurement of circulating immunoglobulin and complement levels. Dipstick urinalysis was also performed. Serum IgE levels were measured in atopic patients using a double antibody radioimmunoassay.[16] Where possible, virus was typed during infections after isolation in Vero cells using monoclonal antibodies specific for HSV-1 and HSV-2 and directly conjugated to FITC (Microtrak, Syva Co., Palo Alto, CA, U.S.A.).

Preparation of antigens

A clinical isolate of HSV-1 was cultured in Vero cells which were harvested 6 or 24 h post-infection, for preparation of CMI or ELISA antigen respectively, as described previously.[17]

Uninfected Vero cells were similarly extracted to act as control antigen. Protein content was measured by the method of Lowry.[18]

Lymphoproliferative assay

Peripheral blood mononuclear cells (PBMN) were purified from fresh heparinized blood by centrifugation on Lymphopaque (Nyegaard Ltd., Birmingham, U.K.). The cells were washed three times and resuspended at 1×10^6 cells/ml in RPMI 1640 medium (Gibco Ltd., Paisley, U.K.) containing 15% autologous plasma, 2 mmol/l L-glutamine, 5 mmol/l 2-mercaptoethanol, and 100 IU/ml penicillin, 200 μg/ml streptomycin, 100 μg/ml gentamycin and 20 μg/ml fungizone. Quintuplicate 200 μl samples were plated onto 96-well flat bottomed microtitre plates (Falcon) in the presence of 10 or 30 μg/ml HSV or control antigen and incubated in 5% CO_2 at 37°C in a humidified incubator for 5 days before being pulsed for 20 h with 0·75 μCi ^{3}H-thymidine (Amersham, Little Chalfont, U.K.) per well. Cells were harvested onto glass fibre discs using an automatic harvester and counted for 1 min in a toluene-based scintillant. Stimulation indices (SI) were calculated as:

$$\mathrm{SI} = \frac{\text{mean counts/min(cpm) with HSV antigen}}{\text{mean cpm with control antigen}}$$

Standard errors for cpm were less than 10% of the mean cpm, and an SI of greater than 3 was considered to be a positive response. A parallel test with concanavalin A (Sigma UK Ltd, Poole, U.K.) at 5 μg/ml as a T cell mitogen was also included as an index of general cell-mediated immunity.

Removal of CD8 +ve cells by panning

Sterile plastic tissue culture grade petri dishes (35 × 10 mm; Primaria, Falcon, Oxnard, U.S.A.) were coated with filter-sterilized CD8 monoclonal antibody (Dako T8, Dakopatts, High Wycombe, U.K.) diluted 1:10 in sodium carbonate/bicarbonate buffer pH 9·6 as used for ELISA[17] at 4°C overnight. Fresh PBMN (3×10^6 in 400 μl medium) prepared as above were incubated on the plates for 2 h at 4°C. The non-adherent cells were washed off with 0·01 mol/l phosphate buffered saline (PBS) pH 7·2 containing 10% foetal calf serum, resuspended at 10^6 cells/ml and used in the lymphoproliferation assay. The presence of CD8 +ve cells in the adherent fraction and their depletion from the non-adherent fraction was confirmed by flow cytometry.

Fluorescent flow cytometry

The method used has been described previously.[19] Briefly, purified PBMN were washed, centrifuged (150 ***g*** for 10 min) and stained with monoclonal antibodies to label either all T cells (UCHT-1, Unipath, Bedford, U.K.), CD8 +ve or CD4 +ve T cell subsets (Dako T8 or T4, respectively). After incubation on ice for 30 min, PBMN were again washed and incubated on ice for 30 min with sheep anti-mouse IgG $F(ab')_2$ conjugated with FITC (Sigma). Cells were washed twice, resuspended at 5×10^5 cells/ml in cold PBS, fixed with 1% formaldehyde, and approximately 10^4 cells analysed on a Coulter EPICS ‘C’ flow cytometer (output 100 mW at 488 nm, flow rate 400–500 cells/s).

ELISA measurement of antibody

The ELISA was based on that described by Booth *et al.*[17] with HSV or control antigen being used to coat Immunoplates (Nunc, Gibco Ltd, Paisley, U.K.) at 10 μg/ml protein at 4°C overnight. The titre was estimated as that dilution of serum which gave absorbance greater than the mean plus 3 standard deviations of five wells of negative pooled serum at 1:100 dilution. HSV specific IgE antibodies were measured using a similar ELISA with anti-human IgE conjugated to horseradish peroxidase (SeraLab, Crawley, U.K.).

RESULTS

Patients

Six patients were thought to have primary HSV infections and 53 complained of recrudescent facial HSV. Nine patients had atopic eczema and, of these, seven had EH. In three patients the EH was particularly severe and affected more than 25% of the body surface area (as calculated by the 'rule of nines'). One patient had recurrent erythema multiforme in association with recrudescent HSV and another suffered disseminated HSV in association with Darier's disease.[20]

HSV-1 was isolated from 22 patients during recrudescences and HSV-2 from four. None of the routine haematological, biochemical, immunoglobulin, complement or urinary investigations yielded results outside the normal range.

Patients with normal CMI to HSV

Fifty six of the 59 patients but none of the 13 controls had positive CMI to HSV (SI > 3). However, all subjects had high lymphoproliferative responses to concanavalin A regardless of HSV status.

Thirty patients with recrudescent HSV and normal CMI were followed for several months. A set of typical results, for Patient 1 who was followed through four successive recrudescences over 2 years, is shown in Table 1. It may be seen that during each recrudescence, the SI was low, it rose to a peak between 1 month and 6 weeks after each recrudescence and then declined slowly over 4–6 months; however, CMI remained positive (SI > 3) throughout. Stimulation indices could not be compared between patients, but fluctuated in 29 patients with the clinical course of their HSV in a similar manner to Patient 1.

In two patients with normal CMI to HSV (Patient 1 and one other patient), depletion of CD8 +ve cells by panning was carried out during recrudescences. In the case of Patient 1 the SI was increased from 4·8 to 12·4 after CD8 depletion and in the other patient the SI increased from 7·3 to 24·9 and the CD4/CD8 ratio from 1·9 to 3·3 in the depleted cell population after panning with CD8 (data not shown).

All patients with a history of HSV infection had circulating antibodies to HSV and in 53 of 59 titres were high (> 1:1600) by ELISA, regardless of the frequency of recrudescences or time since the last attack. Seroconversion from negative (< 1:100) to positive titres was documented in two patients from whom sera predating primary HSV was available. In four patients with primary HSV the antibody levels were low (< 1:1600) for several months after the infection. Two patients with recrudescent HSV also had low antibody titres, but normal CMI to HSV.

All four patients from whom HSV-2 was isolated had positive CMI and antibody titres to HSV-1.

TABLE 1. Variation of CMI (SI) and antibody titre to HSV after three recrudescences over a 2-year period in a normal patient (Patient 1)

Recrudescent lesion	Time since recrudescent lesion	SI	Antibody titre
June 1985	1 day	5·0	1:6400
	2 weeks	13·0	1:6400
	6 weeks	16·0	1:6400
Oct 1986	2 weeks	17·4	1:3200
	1 month	20·4	ND
	3 months	18·4	ND
	4 months	8·2	2:3200
	5 months	3·4	ND
April 1987	1 month	15·2	ND
	6 weeks	22·1	1:6400
	8 weeks	5·5	1:6400
July 1987	3 days	4·8	ND
		(T8 depleted 12·4)	
	1 week	7·6	ND
	2 weeks	8·8	ND

ND = not done

Patients with depressed CMI to HSV

The three patients with prolonged negative CMI (SI < 3) to HSV had all suffered from severe EH involving more than 25% of the skin surface area (Table 2).

In Patient 2, EH was caused by a primary attack of HSV which was confirmed by the negative ELISA (< 1 : 100) on two serum samples collected before and one collected during the attack. The HSV antibody titre subsequently rose to > 1 : 3200, but despite this, the patient's CMI remained < 2 for 16 months. Nine months after EH, CD8 + ve cell depletion (CD4/CD8 ratio increased from 1·9 to 3·2 in the depleted cell population after panning with DAKO T8) produced an increase in SI from 1·8 to 3·2; when repeated 3 months later the SI was again increased from < 1 to 2·1.

Patient 4 also suffered a primary attack but the ELISA titre remained low (1 : 200 after 1 week; 1 : 800 after 4 months; 1 : 400 after 6 months) and the SI was < 2 initially. When determined 6 months after EH the SI was 1·7 and depletion of CD8 + ve cells from a PBMN cell suspension (CD4/CD8 ratio increased from 2·1 to 3·1) failed to increase the SI much further (2·2 after depletion). Eight months after EH the SI was still below 3 and CD8 + ve cell depletion did increase this from 1·32 to 2·3.

Patient 3 had low CMI to HSV (SI < 3) for 6 months following EH. After this the SI remained within the normal range (SI > 3) for the next 6 months until it was measured again 2 weeks after a mild recrudescence when it had fallen to 2·05. The EH was due to recrudescent rather than primary HSV and the patient's HSV antibody titres measured in two pre-EH stored sera were 1 : 6400, a level which remained constant throughout the recrudescence. Like all other patients, Patient 3 had a normal lymphoproliferative response to concanavalin A.

All three of these patients were very ill and pyrexial at the time of their EH and required hospitalization. However, their infections responded rapidly to oral acyclovir (200 mg five times

TABLE 2. The cell mediated immunity, as stimulation index (SI) and ELISA antibody titre to HSV in eczema herpeticum patients with >25% surface area involvement

	Time	SI	ELISA titre
Patient 2	Pre lesion serum	ND	<1:100
	Eczema herpeticum	1·1	<1:100
	Post lesion: 1 week	<1	ND
	2 months	<1	1:3200
	6 months	<1	1:3200
	8 months	<1	1:6400
	9 months	1·8	1:3200
		(T8 depleted 3·2)	
	11 months	<1	
	12 months	<1	1:1600
		(T8 depleted 2·1)	
	14 months	<1	1:1600
Patient 3	Pre lesion serum	ND	1:6400
	Eczema herpeticum	2·3	<1:6400
	Post lesion: 1 week	1·6	1:6400
	5 months	2·15	1:6400
	6 months	5·4	1:6400
	8 months	28·9	1:6400
	12 months	2·05	1:6400
	(2 weeks after cold sore)		
Patient 4	Pre lesion serum	ND	ND
	Eczema herpeticum	ND	ND
	Post lesion: 1 week	1·83	1:200
	4 months	0·83	1:800
	6 months	1·74	1:400
		(T8 depleted 2·2)	
	8 months	1·32	1:400
		(T8 depleted 2·3)	

ND = not done

daily for 7 days). There were no recurrences of EH and all three patients had remarkably good control of their eczema since their EH resolved, none of them requiring more than occasional dilute steroid applications and emollients.

The four EH patients with normal CMI to HSV had antibody titres >1:1600, were less extensively affected than the three with depressed CMI (<25% body area) and did not require hospitalization. The two atopic patients with recurrent cold sores also had normal CMI and antibody responses to HSV. There was no association between the state of the eczema and the severity of herpetic infection, nor with the immune response to HSV which, in turn, was not clearly related to the total circulating IgE level. The IgE level varied considerably between patients (29–63,000 IU/ml). No specific IgE antibody to HSV was detectable by ELISA.

DISCUSSION

Twenty three of the patients reported histories of family or household contacts with HSV, an early age of onset of the lesions (mean 14 years; range infancy to 50 years) and several common triggering factors for their recrudescences including frost, sun exposure, stress, menstruation and infections. No unexpected underlying diseases were discovered as a cause for abnormal immunity to HSV in patients suffering from frequent recrudescences. Indeed, all but three patients with a history of HSV infection, but none of the controls, had positive antibody titres and CMI to HSV, regardless of the frequency of, or time since, recrudescent HSV. The three notable exceptions, with prolonged depression of CMI, had all suffered from extensive EH.

In the 30 patients with normal CMI to HSV investigated on several occasions, depression of CMI was seen during attacks, with a peak activity (SI) 4–6 weeks later, and a slow decline to a background positive level thereafter; these results are similar to those reported by some authors,[8–10] but contrast with those of others who suggested that peak activity occurred during recrudescences.[4,11]

Our results would fit with the hypothesis of a temporary deficit in CMI to HSV allowing a recurrence to become a clinical recrudescence. The 4–6 week delay before peak lymphoproliferation may be due to CD8 mediated virus-specific suppression of immunity.[6,13,15] This is supported by the improvement in SI produced during recrudescence after CD8 +ve lymphocyte depletion.

Anergy and impaired *in vitro* CMI to several antigens have been recorded in atopic patients[21,22] and the high IgE level often seen in this condition has been attributed to T suppressor dysfunction. There is evidence of a deficiency in unidentified circulating IgE specific suppressor factors in serum from atopic patients.[23] Both hyper-[24] and hypo-[25] activity of NK cells from atopic eczema patients have been described and, together with impaired CMI[23,24] and interferon production, have been suggested as mechanisms of the well known sensitivity of atopic patients to many different infectious organisms that particularly affect the skin.[26] Leung *et al.*[27] recorded reduced cytotoxic/suppressor (CD8+) cell numbers and function as well as impaired helper/inducer (CD4+) T cell activity in mixed lymphocyte cultures, in patients with atopic eczema. Our results suggest that prolonged CD8 suppression produced the observed defect in CMI to HSV in the three patients most severely affected with EH.

Several authors[21–26] have recorded an association between the immunological abnormalities of atopic eczema patients, the overall state of their eczema and their circulating IgE concentrations. However, the EH patients recorded here with defective CMI to HSV exhibited no obvious difference, in either their serum IgE level or the activity of their eczema, from the four more mildly affected EH patients and the two atopic eczema patients who suffered from cold sores. These differences from the patterns noted by others might be due to the small numbers of our EH and atopic eczema patients, but Rystedt *et al.*[26] also noted a lack of association between the activity of atopic eczema and the sensitivity to infections.

A recent report[28] described deficient mixed autologous cell reactions to Langerhans cells and reduced ETAF production by epidermal cells from atopic patients despite normal Langerhans cell induced proliferation to HSV. Defective interleukin production was thought to contribute to the abnormal local immune response to HSV in atopic skin. We have preliminary evidence that, during the time of suppressed systemic CMI to HSV, local CMI is not impaired to the same extent.

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Effect of UVB therapy and a coal tar bath on short contact dithranol treatment for psoriasis

Y.PARAMSOTHY, M.COLLINS* AND C.M.LAWRENCE

Department of Dermatology and *Department of Community Medicine, North Staffordshire Hospital Centre, Stoke-on-Trent, U.K.

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SUMMARY

The effect of UVB therapy after short contact dithranol therapy (SCDT) was examined in 53 patients with psoriasis. After dithranol application, patients were randomly allocated to receive either a tar bath and UVB therapy (27 patients) or an emulsifying ointment bath only (26 patients). Forty eight patients completed the study; 16 of the 21 dithranol only patients cleared in a mean of 19·5 days compared with 20 of the 27 dithranol+UVB patients who cleared in a mean of 20·3 days. These differences were not significant. Similarly there was no significant difference in response between the dithranol only and dithranol UVB group as measured by the change in plaque thickness measured using Harpenden calipers, and the severity of psoriasis assessed by the extent of the rash, the degree of scaling, redness and induration. Thirteen of the 16 dithranol only patients who cleared relapsed in a mean of 10·6 weeks compared with 14 of the 20 dithranol UVB patients who relapsed after a mean of 18·9 weeks ($P < 0{\cdot}05$). Comparison of pre- and post-treatment full blood count, biochemical screen and urinalysis showed no evidence of systemic toxicity due to SCDT. This study shows that UVB therapy does not improve the clearance of psoriasis in SCDT, but does significantly postpone relapse.

Short contact dithranol therapy (SCDT) is an effective treatment for chronic stable plaque psoriasis.[1,2] It is more convenient than the Ingram regime[3] and causes less skin staining, although inflammation of surrounding normal skin remains a serious problem.[4] A coal tar bath and ultraviolet (UV) irradiation therapy have always formed an integral part of the Ingram regime despite the inconclusive evidence of their effectiveness.[5,6] More recent work[7] using a very small area of psoriasis suggests that combining dithranol with UV therapy increases the rate of clearing of psoriasis provided a UV source that does not emit UVC is used. The present study was designed to test these findings in a clinical situation by comparing the effect of whole body UVB therapy after SCDT with SCDT alone, using a UV source that contained no UVC.

Correspondence: Dr C.M.Lawrence, Department of Dermatology, The Royal Victoria Infirmary, Newcastle upon Tyne NE1 4LP, U.K.

METHODS

Patients

Fifty three patients (21 females and 32 males; mean age 44·8 years, range 16–88 years) with stable chronic plaque psoriasis requiring in-patient treatment and who were willing to take part in the trial were studied. Patients were divided into those with 35% or less and those with 36% or more of the body surface involved by psoriasis. All patients received SCDT. Patients were randomly assigned, using permutated block structure of 4, to receive either a coal tar bath (50 ml coal tar solution BP added to the bath water) and UVB, or an emulsifying ointment bath, after their SCDT. At the start of the study there was no significant difference between the two groups. The 26 patients (17 male, nine female, mean age 42·5 years) in the dithranol only group had 28% of their body surface area involved by psoriasis, mean disease duration 7·6 months, compared with the 27 patients (15 male, 12 female, mean age 46·9 years) in the dithranol UVB group who had 30·8% of their body covered by psoriasis, mean disease duration 6·1 months. A full blood count, biochemical screen and urinalysis were carried out on all patients at the start and end of the study.

Dithranol

Dithranol was applied in emulsifying ointment with 0·5% salicylic acid at concentrations of either 1, 2, 4, 8, 12 or 16%, left on the plaques for 20 min and then washed off, using Teepol shampoo if necessary.[8] All patients started with 1% dithranol; the concentration was increased every second or third day if there was no inflammation of the surrounding skin.

UVB therapy

After SCDT, the 27 patients randomized to receive UVB therapy had a coal tar bath followed by UVB therapy 5 days a week. UVB treatment was given by a trained physiotherapist using a Waldmann UV 1000 cabin fitted with 26 Waldmann F85/100W/UV6 fluorescent lamps with an irradiance of 0·5 mW/m^2. This lamp had an emission spectrum of 285–350 nm, maximal at 310–315 nm (Fig. 1). The dose required to produce minimal erythema was assessed in 15 patients. From these results a starting dose of half the minimal erythemal dose was given depending on skin type so that the five patients with skin type I[9] received a starting dose of 15 mJ/m^2 (30 s), the five with skin type II received 21 mJ/m^2, the eight with skin type III received 26 mJ/m^2 and the nine skin type IV patients received 31 mJ/m^2. The exposure time was increased by 30% on successive days so that the patient received a dose sufficient to cause slight redness without burning.

Other therapy

Any inflammation caused by dithranol was treated with topical 0·25% fluocinalone acetonide BP (Synalar® cream) for 2–3 days. Emulsifying ointment was used as an emollient and areas not suitable for SCDT such as flexures, scalp or face were treated with a variety of other topical agents. These areas were excluded from the study.

Assessment

Weekly visual assessment of extent of psoriasis was made using the rule of nine. Scaling, redness and induration were also assessed clinically and scored on a scale from 0–4. In 22 patients with forearm plaques, weekly plaque thickness measurements were made using Harpenden calipers.[10] Clearance was defined as the point at which the patient had less than 3% of their skin

TABLE 1. Number of patients who cleared, failed to clear or withdrew from each treatment group

	No. of patients			
	Cleared	Failed to clear	Withdrew	Total
SCDT	16	5	5	26
SCDT + UVB	20	7	0	27
Total	36	12	5	53

involved by psoriasis. Patients who failed to show definite improvement after 21 days were withdrawn from the study and considered as treatment failures. Patients who cleared were reviewed regularly and relapse was considered to have occurred if the area of the skin affected by psoriasis reached half the area that had been affected at the start of the study.

Statistical analysis

Paired and unpaired *t*-tests and the chi-squared test were used for statistical analysis. Values presented are means ± 95% confidence intervals.

RESULTS

Five patients from the dithranol only group withdrew from the study. Of the 48 patients who completed the trial 36 cleared, including 16 patients from the dithranol only group and 20 patients from the dithranol UVB group. Twelve of the 48 patients who completed the study failed to clear. There was no significant difference between the numbers of patients who cleared in the two groups ($P > 0{\cdot}05$; χ^2 test) (Table 1).

The maximum concentration of dithranol used was significantly lower in patients who cleared than in those who failed to clear (9·3 ± 1·2% and 12·2 ± 1·5%, respectively; $P < 0{\cdot}05$, unpaired *t*-test).

Clearing rate

There was no significant difference between the mean number of days to clear in the dithranol only group (19·5 ± 2·6 days, range 12–30 days) and the dithranol UVB group (20·3 ± 1·6 days,

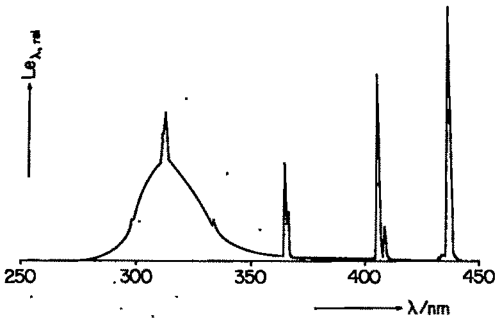

FIGURE 1. Emission spectrum of the UVB source (F75/85W1UV6) used in the study.

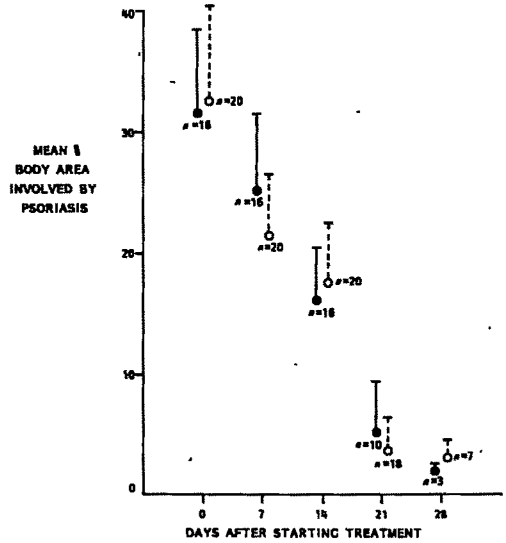

FIGURE 2. Extent of the rash in the 20 patients who cleared with dithranol UVB (○) and the 16 patients who cleared with dithranol alone (●). Points are means ± 95% confidence intervals.

range 12–28 days). Weekly assessments of the extent of the rash (Fig. 2) and the scores for redness, scaling and induration showed no difference between the patients who cleared in the two groups. Objective measurement of the rate of clearance of the rash using weekly measurement of plaque thickness also showed no significant difference between the two groups

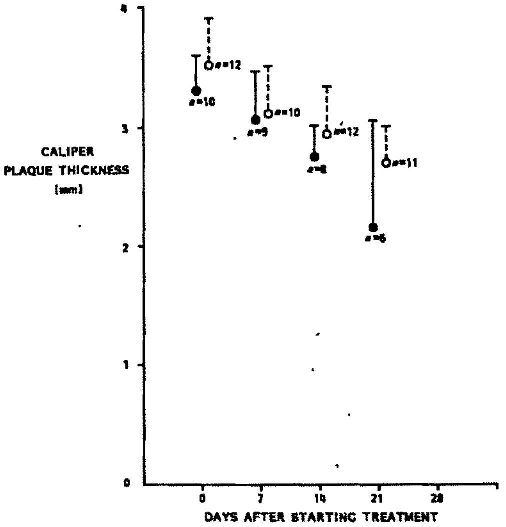

FIGURE 3. Forearm plaque thickness in 12 of the patients who cleared in the dithranol UVB group (○) and 10 of the patients who cleared in the dithranol only group (●). Points are means ± 95% confidence intervals.

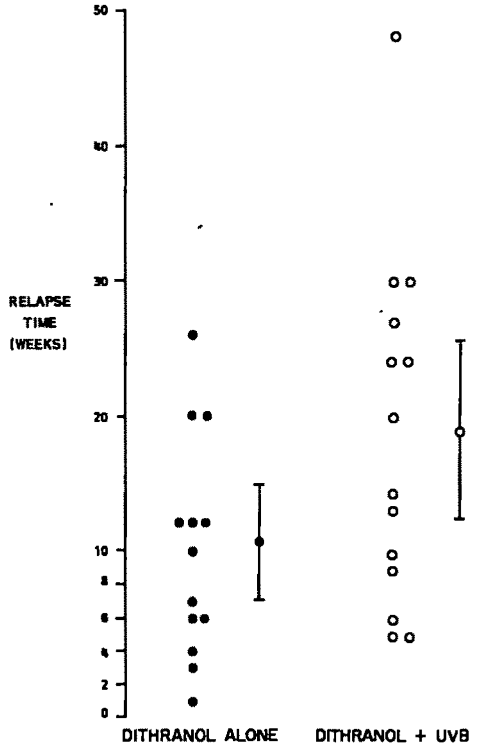

FIGURE 4. Relapse time in 13 patients treated with dithranol only (●) and 14 patients treated with dithranol + UVB (O). Bars indicate means ± 95% confidence intervals.

(Fig. 3) and there was no significant difference between the mean concentration of dithranol used by the two groups (8·8 ± 1·2% and 9·7 ± 1·2%, respectively).

UVB therapy

There were no significant differences between the mean starting erythemally effective UVB dose (clearers 24 mW/m^2, non clearers 26 mW/m^2), final dose (clearers 101 mW/m^2, non clearers 105 mW/m^2) or cumulative dose (clearers 707 mW/m^2, non clearers 764 mW/m^2) or the total number of UVB treatments (clearers and non clearers 12) received by the 20 patients who cleared and the seven patients who failed to clear. A maximal phototherapy dose was given to our patients, as judged by the soreness and erythema that the patients developed. All 20 of the UVB treated patients who subsequently cleared, developed erythema on at least one occasion with a mean of three episodes of erythema (range 1–8), and soreness or burning occurred in 12 patients. In the seven patients who failed to clear after phototherapy, erythema occurred in five patients with a mean of 4·4 episodes of erythema (range 2–8). The two patients in whom erythema was not recorded developed soreness on one and three occasions, respectively.

Laboratory investigations

Pre- and post-treatment biochemical, haematological and urinalysis data was available in 37 of the 48 patients who completed the study. There were no significant changes, with the exception of a fall in the mean alkaline phosphatase activity of 9 units which is of no clinical significance.

Two patients who had a normal pre-treatment urinalysis, showed a trace of albumin post-treatment, one of these had also developed a urinary tract infection.

Relapse rate

Regular follow up was carried out on all 36 patients who cleared. In the dithranol only group, 13 of the 16 patients relapsed in a mean time of 10·6 weeks (range 1–26 weeks) and three remained clear after a mean of 42 weeks (range 21–69 weeks). In the dithranol UVB group 14 relapsed after a mean of 18·9 weeks (range 5–48 weeks) and six remained clear for a mean of 30·8 weeks (range 23–39 weeks). These differences were statistically significant ($P < 0{\cdot}05$) (Fig. 4).

DISCUSSION

This study shows that the addition of UVB therapy, using a light source with no UVC output, significantly increased the length of remission after SCDT, but did not increase the number of patients who cleared or alter the time to clear. Farr *et al.*[7] demonstrated that UVB phototherapy using a Helarium lamp, which produces an emission spectrum similar to that produced by the equipment used in the present study, reduced the time required to clear forearm plaques of psoriasis, although they did not measure the relapse time. Since only forearm plaques were treated it is possible that they used a UVB dose above that which could be used when the whole body is treated, since it is impossible to give a uniform dose to all body surfaces. In the present study, the dithranol UVB group were not cleared to a greater extent than the dithranol only group as judged by the measurements of plaque thickness or disease extent and severity. Although a different therapeutic response might have been expected if a greater exposure time had been used, it appears that our patients received a maximal UVB dose as judged by the erythema and soreness, one of which developed at least once in all our patients. Other workers who added SCDT to a phototherapy regime also failed to demonstrate any enhanced clearing of psoriasis, but they used a fluorescent sun lamp[11,12] which emits some UVC and this may prevent an adequate therapeutic effect due to burning.

Thus, the study has shown that the addition of UVB to SCDT does not reduce the clearance time or increase the number of patients who clear, but does prolong the duration of remission after clearance and thus remains a useful adjunct therapy in the management of patients using SCDT.

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The role of psoralen photochemotherapy (PUVA) in the treatment of severe atopic eczema in adolescents

D.J.ATHERTON, F.CARABOTT,* M.T.GLOVER AND J.L.M.HAWK*

Departments of Paediatric Dermatology and *Photobiology, St. John's Hospital for Diseases of the Skin, Lisle St., London WC2H 7BJ, U.K.

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SUMMARY

Fifteen adolescent children with severe, persistent atopic eczema were treated with oral psoralen photochemotherapy (PUVA). This resulted in initial clearance of eczema in 14 of the 15 children, nine of whom achieved a remission. Apart from its effectiveness, a major benefit of this therapeutic approach was that it was associated with resumption of normal growth in children who were previously growing poorly, either as a direct result of severe eczema or its treatment. Nevertheless, against the considerable advantages of PUVA for this group of patients have to be balanced the possible hazards, because relatively high exposures are required in some individuals, both initially to induce clearance and subsequently to maintain it.

Severe persistent atopic eczema in older children and adolescents currently presents one of the greatest therapeutic challenges in dermatology. An additional problem in the management of these patients is the frequent presence of associated growth failure. The aetiology of this growth failure is not well understood, but it seems likely that it is contributed to both by the disease itself and, in many cases, by topical or systemic treatment with corticosteroids.[1] These children, therefore, require therapy which is both effective and which allows the resumption of normal growth before the end of the growth period. Many patients with atopic eczema who live in northern countries regularly experience an improvement in the condition during the summer months or find that a vacation in a sunny climate may be extremely beneficial. For these reasons, artificial ultraviolet (UV) irradiation has been used in the treatment of atopic eczema, with reports of benefit from both UVB and from combined UVA plus UVB.[2,3] However, like Morison *et al.*,[4] we have not found UVB treatment beneficial in severe disease, and we have no experience of combination treatment. Following the report of successful treatment of atopic eczema in adults by oral psoralen photochemotherapy with UVA (PUVA),[4] we were encouraged to use this approach in older children and adolescents with exceptionally severe, persistent disease not responding adequately to other forms of treatment, particularly when accompanied by significant growth retardation.

Correspondence: Dr D.J.Atherton.

METHODS

Over a period of 4 years, we accepted for PUVA treatment a total of 15 children, all of whom had long-standing, severe atopic eczema which had proved refractory to other forms of treatment. No child was accepted for treatment in the absence of significant handicap. Most of them had become unable to attend school because of the severity of their eczema. All the children had universal or virtually universal atopic eczema, which was of the erythrodermic type in most cases. Eight were boys; seven were girls. Their median age at the start of treatment was 13·6 years (range 10·1–14·7 years); 14 were of skin types I–IV and one was of skin type VI.[5]

The heights of 10 of the 15 children were on or below the 3rd centile at the start of treatment. All these 10 children either had asthma (eight children), or had received oral corticosteroids for the treatment of eczema or asthma (eight children). All 10 had been treated for many years with topical corticosteroids. We were able to obtain sufficient previous growth data in seven of these 10 children to determine their growth velocity prior to treatment. Six of the seven had growth velocities on or below the 3rd centile, and one had a growth velocity on the 10th centile.

The children were given oral 8-methoxypsoralen at a dose of 0·6 mg/kg body weight, 2 h before UVA exposure. Nine were initially treated three times weekly; the other six were treated twice weekly. Because of the extent of these patients' skin disease, it was not possible to use irradiation tests to determine the appropriate initial UVA treatment dose; this was therefore a standard 1 J/cm^2. The UVA dose was gradually increased by increments of 0·5–2·0 J/cm^2, at intervals of not less than a week, depending on skin tolerance and response, until clearance or near-clearance occurred. In some cases, because of intense irritability of the skin, it was difficult to increase UVA exposure adequately. However, we were able to overcome this problem by the use of short courses of oral prednisolone, the dose being tapered off gradually and withdrawn in 8–12 weeks.

After clearance, maintenance therapy was established, during which the frequency of treatments was gradually decreased, the dose administered on each occasion remaining unaltered. The aim during this phase was to be able, after a period, to withdraw PUVA therapy altogether, without this resulting in relapse of disease, i.e. to achieve a state of remission. The organization of treatment is illustrated in Figure 1.

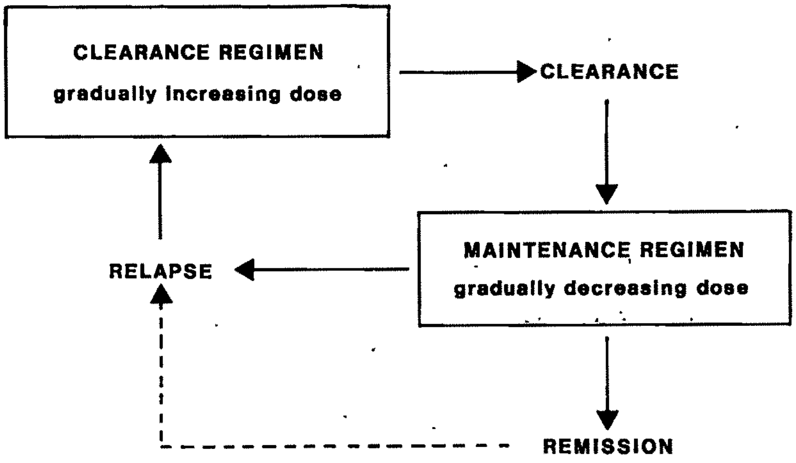

FIGURE 1. Diagram showing the organization of the different phases of treatment.

RESULTS

Initial clearance or near-clearance was achieved in 14 of the 15 patients, the exception being a 10-year-old boy who elected to discontinue treatment because he could not tolerate the heat inside the cabinet. Time to clearance in the other 14 patients ranged from 12–25 weeks (median 16 weeks). At the time of clearance, UVA dose/treatment ranged from 2·0–15·0 J/cm^2 (median 9·0 J/cm^2), and the cumulative UVA dose ranged from 50–590 J/cm^2 (median 155 J/cm^2). Five children had received concurrent short courses of oral prednisolone to permit the necessary

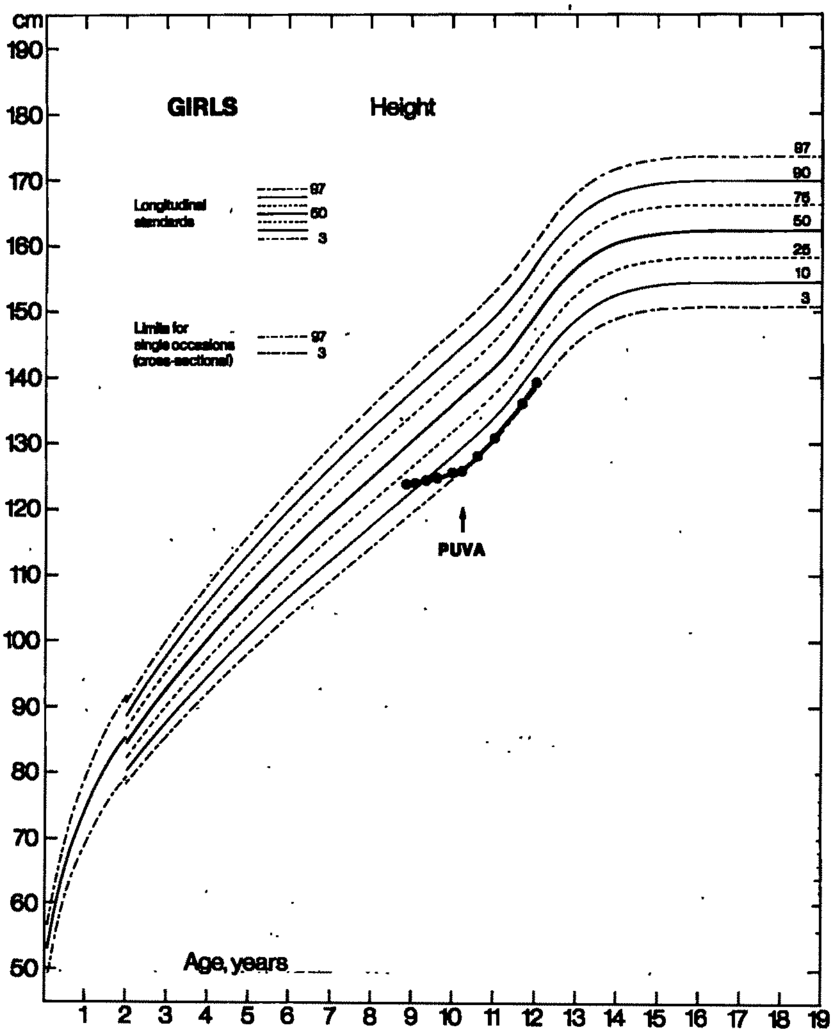

FIGURE 2. Growth chart, showing the improvement observed in the growth of a girl with severe atopic eczema in whom PUVA was started at the age of 10 years. Prior to PUVA, she did not have a significant degree of asthma, and her eczema was being treated with no corticosteroids other than topical 1% hydrocortisone ointment.

increases in UVA exposure. At the time of clearance, no patients were still using topical or systemic corticosteroids.

At the time of writing, complete withdrawal of PUVA without relapse (i.e. remission), had been achieved in nine children. In six of these, the remission was achieved following a single clearance phase. In one child, relapse occurred during the first maintenance phase, and remission followed a second clearance and maintenance phase; in the other two, relapse occurred during two successive maintenance phases, and remission followed the third. Time from the start of treatment to remission ranged from 0·3–1·8 years (median 1·0 years). Cumulative UVA dose at remission ranged from 51–1307 J/cm^2 (median 371 J/cm^2) in the seven patients with skin types I–IV, and was 2114 J/cm^2 in the patient with skin type VI. At the time of writing, the duration of remission was 0·25–4·2 years (median 1·1 years). Remission exceeding 1 year has so far been observed in five children, none of whom now regularly require any skin treatment. However, as is the experience of many who have in the past been subject to severe atopic eczema, three of the five have occasionally experienced minor recurrences of eczema which they have cleared by short-term application of emollients and mild topical corticosteroids.

Of the remaining five children, who were not in remission, two were currently in the maintenance phase, but the other three patients discontinued treatment in relapse. These three had all required adjunctive oral corticosteroids to achieve clearance initially, and had relapsed during the maintenance phase. We decided to discontinue PUVA as we were unwilling to use oral corticosteroid therapy again and did not believe that we would achieve a second clearance without doing so. These three patients had already had cumulative doses of 350, 1740 and 1973 J/cm^2, respectively.

Of the seven children for whom adequate previous growth data were available, the six whose growth velocities had been below the 3rd centile showed accelerated growth during and after treatment (Fig. 2). Five of these achieved growth velocities on or above the 97th centile, and the sixth a growth velocity between the 50th and 97th centiles. Four of these six were in remission at the time of writing.

In general, PUVA was well tolerated as long as dose increments were sufficiently small and were made very gradually. The relatively high temperature inside the PUVA cabinet was a problem for several of the children, but one which could largely be overcome by allowing one or more breaks out of the cabinet during treatment.

Complications of therapy included a limited outbreak of cutaneous herpes simplex in one child, photo-onycholysis in one child and development of typical PUVA freckles in three. None of these complications necessitated discontinuation of treatment, although two of those who developed freckles were among the three who discontinued treatment in relapse.

DISCUSSION

It is our impression that PUVA offers a very effective form of therapy for some of the most difficult cases of refractory atopic eczema in older children and adolescents. It has proved very acceptable to such patients and has, therefore, been associated with a remarkably high level of compliance, something that is often difficult to achieve in severely eczematous children in this age group. We have been very gratified by the high rate of initial clearance, and by the relatively frequent achievement of remissions, many of which have been prolonged. Although one might have expected one or more of these children to enjoy spontaneous improvement during the period of this study, our experience suggests that in practice this is a relatively unusual occurrence in children of this age who are as severely affected as these. Whereas a placebo effect is certainly likely to have occurred in this group of children, the degree and duration of benefit experienced by most of them makes it highly improbable that this was the principal reason for their improvements.

Apart from the high response rate, and the relatively frequent induction of remission of disease, the other great benefit of PUVA in this group of children was its effect upon growth. PUVA did, at the very least, allow these children to resume a normal rate of growth. Very high growth velocities were observed in six children during treatment. In four of these, this probably represented the onset of the pubertal growth spurt, which the evidence suggests had been delayed, probably by a combination of the disease itself and the growth-retarding effects of drugs used in therapy, particularly corticosteroids.[1] These children were likely to have failed to demonstrate a normal pubertal growth spurt had they remained on their previous, ineffective treatment regimens. In the other two children, the growth acceleration occurred prior to the onset of puberty.

Our main anxiety with PUVA treatment of these patients relates to the relatively high UVA exposures required in a few of them, both to induce clearance and to maintain it subsequently. Although the long-term hazards of PUVA in children have not yet been clearly defined, suggestions of an increased incidence of skin malignancy[6] have been questioned,[7] and in our view, the benefits of PUVA greatly outweigh its currently established disadvantages in this particular group of patients.

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An epidemiological comparison between hand eczema and non-hand eczema

C.L.GOH

Contact Clinic, Middle Road Hospital, Singapore

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SUMMARY

Thirty four per cent of 2110 patients with eczema attending a contact dermatitis clinic presented with hand eczema. An epidemiological comparison was made of patients with hand eczema and non-hand eczema (defined as eczema on parts of the body other than the hands). Occupational eczema was significantly more common in the hand eczema than the non-hand eczema group ($P = 0.0011$). The prevalence of atopy was the same in both groups (13%). The prevalence of irritant contact dermatitis was higher in the hand eczema group (32%) than the non-hand eczema group (13%) ($P < 0.0001$). The rate of allergic contact dermatitis was lower in the hand eczema group (23%) than the non-hand eczema group (39%) ($P < 0.0001$). The rate of positive patch test reactions was lower in the hand eczema group (41%) than the non-hand eczema group (56%) ($P < 0.0001$). Nickel sulphate (8%), cobalt chloride (3%), potassium dichromate (3%), and fragrance mix (4%) were common allergens encountered in hand eczema group. None of these allergens was specifically more prevalent in the hand eczema than the non-hand eczema group. However, the prevalence of allergy to nickel, colophony, epoxy resin and medicaments was significantly higher in the non-hand eczema group.

Hand eczema is a common dermatological condition. The management of hand eczema depends on its cause. While allergic or irritant contact dermatitis of the hand can usually be elucidated by proper history taking and patch tests, endogenous hand exzema is often diagnosed after exclusion of the former conditions. Physical examination alone is not helpful in differentiating the cause of hand eczema. No distribution of eczema is typically allergic, irritant or endogenous.[1]

The aim of the present study was to identify epidemiological differences between patients with hand eczema and those with non-hand eczema (defined as eczema on parts of the body other than the hands).

METHODS

All patients attending the contact dermatitis clinic of Middle Road Hospital, Singapore, with eczema, between January 1985 and December 1986 were included in the study. The patients

Correspondence: Dr C.L.Goh, Contact Clinic, Middle Road Hospital, Middle Road, Singapore 0718.

were patch tested with the International Contact Dermatitis Research Group (ICDRG) standard series, and additional allergens where indicated, with Finn Chambers and Scanpor tape. Reading and recording of patch test reactions was performed according to the method described by Cronin.[2] The age, sex, history of atopy, patch test results and diagnosis (and relevant occupational details) were recorded. 'Hand eczema' was defined as eczema confined to the hands (up to the wrist line). 'Non-hand eczema' was defined as eczema on parts of the body other than the hands. A patient with co-existing eczema of the hands and other parts of the body was regarded as belonging to the 'non-hand eczema' group.

The diagnosis of allergic contact dermatitis was based on the history and a relevant positive patch test and that of irritant contact dermatitis on the history and negative patch tests. 'Endogenous/unclassifiable eczema' referred to all eczema other than allergic and irritant contact dermatitis, including those with classical endogenous patterns such as atopic and seborrhoeic eczema. It inevitably included some contact dermatitis in which relevant irritant and allergic contact factors were missed. When allergic and irritant contact dermatitis co-existed, it was classified as allergic contact dermatitis. Occupational dermatitis was defined as eczema caused by or aggravated by the working environment. Atopic conditions included atopic eczema, asthma and hay fever.

The chi square test was used for statistical analysis.

RESULTS

Two thousand one hundred and ten patients were included in the study (mean age 33 years, range 6–88 years); 1127(53·4%) were male (mean age 34 years, range 8–88 years) and 983(46·6%) were female (mean age 31 years, range 6–84).

Table 1 shows the prevalence of hand and non-hand eczema in each sex; 721(34%) patients had hand eczema (mean age 32 years, range 8–81 years); and 1389(65·8%) patients had non-hand eczema (mean age 33 years, range 6–88 years).

Table 2 compares the prevalence of occupational dermatitis and atopic history, in each sex, between the hand eczema and non-hand eczema groups. The prevalence of occupational dermatitis was significantly higher in the hand eczema than in the non-hand eczema group. The prevalence of occupational hand dermatitis was significantly higher in males than in females in both groups. There was no significant difference in the prevalence of atopic history between the two groups.

Table 3 compares the prevalence of hand and non-hand eczema according to cause. The difference in the prevalence of allergic contact dermatitis, irritant contact dermatitis and

TABLE 1. The prevalence of hand eczema and non-hand eczema in 2110 patients with eczema

	No. of patients (%)		
	Male	Female	Total
Hand eczema	404 (35·8%)	317 (32·2%)	721
Non-hand eczema	723 (64·2%)	666 (67·8%)	1389
Total	1127 (100%)	983 (100%)	2110

TABLE 2. The prevalence of occupational dermatitis and atopic history in patients with hand eczema and non-hand eczema

	No. of patients (%)				
	Atopic history		Occupational dermatitis		
Hand eczema					
male ($n=404$)	48(12%)		142(35%)	$P=0\cdot0011$	
female ($n=317$)	35(11%)		75(24%)		
total ($n=721$)	83(12%)	NS	217(30%)		$P<0\cdot0001$
Non-hand eczema					
male ($n=723$)	94(13%)		121(17%)	$P<0\cdot001$	
female ($n=666$)	86(13%)		23 (3%)		
total ($n=1389$)	180(13%)		144(10%)		

NS = not significant

TABLE 3. The prevalence of hand and non-hand eczema according to cause

	No. of patients (%)						
	Allergic contact dermatitis		Irritant contact dermatitis		Endogenous/ unclassifiable eczema		Total
Hand eczema [atopy]	162(23%) [20(12%)]	$P<0\cdot0001$	229(32%) [20(9%)]	$P<0\cdot0001$	330(45%) [53(16%)]	$P=0\cdot04$	721(100%)
Non hand eczema [atopy]	487(39%) [57(12%)]		179(13%) [13(7%)]		723(52%) [130(18%)]		1389(100%)
Total	649		408		1053		2110

endogenous/unclassified eczema between the two groups was statistically significant ($P<0\cdot0001$). The prevalence of allergic contact dermatitis and endogenous/unclassifiable eczema was higher in the non-hand eczema group. The prevalence of irritant contact dermatitis was significantly higher in the hand eczema group ($P<0\cdot0001$), and was marginally higher in males (33%) than in females (29%), but this difference was not statistically significant.

Table 4 compares the prevalence of contact allergy to some allergens of the standard ICDRG series in the hand eczema and non-hand eczema groups; 298(41%) patients in the hand eczema group and 727(52%) in the non-hand eczema group were sensitive to one or more patch test allergens ($P<0\cdot0001$). No allergen was specifically more associated with the hand eczema group than with the non-hand eczema group. The prevalence of allergy to nickel sulphate, epoxy resin, and medicaments (proflavine, clioquinol and neomycin) was significantly higher in the non-

TABLE 4. The prevalence of contact allergy to some common allergens in the standard ICDRG series in patients with hand eczema and non-hand eczema

Allergen	Hand eczema ($n=721$)	Non-hand eczema ($n=1389$)	P
Nickel sulphate 5%	54 (8%)	181 (13%)	0·0002
Fragrance mix 8%	25 (4%)	72 (5%)	NS
Cobalt chloride 1%	20 (3%)	56 (4%)	NS
Potassium dichromate 0·5%	19 (3%)	26 (2%)	NS
Proflavine 0·5%	18 (3%)	65 (5%)	0·02
Neomycin 20%	19 (3%)	65 (5%)	0·03
Colophony 20%	16 (2%)	66 (5%)	0·006
Wool alcohol 30%	11 (2%)	35 (3%)	NS
p-phenylenediamine 1%	13 (2%)	40 (3%)	NS
Clioquinol 3%	12 (2%)	46 (3%)	0·04
Balsam of Peru	13 (2%)	32 (2%)	NS
Thiuram mix 3%	7 (1%)	19 (1%)	NS
Epoxy resin 1%	7 (1%)	5 (0·4%)	0·05

hand eczema group; 54% (162 of 298) of patients with hand eczema and 67% (487 of 727) with non-hand eczema had relevant patch test reactions, this difference was not statistically significant.

DISCUSSION

Hand eczema is a common and frequently disabling condition.[2] It accounted for about 30% of patients with eczema in Europe[3] and 21% in the UK.[4] The prevalence in our patients was 34%. It was higher in males (36%) than in females (32%). Hand eczema is often occupational, and occupational dermatitis is more prevalent in males than in females.[5] This might account for the higher prevalence of hand eczema in males. The prevalence of occupational dermatitis was significantly higher in the hand eczema group (30%) than in the non-hand eczema group (10%). Its prevalence was significantly higher in males than in females in both groups.

The prevalence of an atopic history in the hand eczema and non-hand eczema groups was the same. It was low compared with previous reports. Cronin[1] reported an atopic history in 70% of women with hand eczema in the UK and Glickman *et al.*[6] reported an atopic history in 82% of housewives with hand eczema in the USA. One reason for our low prevalence could be unreliable history giving by our patients. Most patients were not familiar with the symptoms and signs of conditions associated with atopy. There was a tendency to relate the cause of all skin disorders to food allergy.

It is often difficult to elucidate the cause of hand eczema. Contact dermatitis often occurs in a patient with endogenous eczema or with a genetic predisposition.[7] The prevalence of irritant contact dermatitis was significantly higher in the hand eczema than the non-hand eczema group, but those of allergic contact dermatitis and endogenous/unclassifiable eczema were lower. Twenty three percent of patients with hand eczema had allergic contact dermatitis. The prevalence was lower than that reported in Swedish women (36%),[8] and in European housewives (42%),[9] but comparable to the prevalence in eczema patients in the USA (26%).[10]

No allergen appeared to be more commonly associated with the hand eczema than the non-hand eczema group. Allergy to nickel, colophony, and medicaments (clioquinol, proflavine and neomycin) appeared to be significantly more prevalent in the non-hand eczema group. The common allergens in the hand eczema group were nickel, cobalt, fragrance mix, chromate and medicaments (proflavine, clioquinol and neomycin). The allergens were similar in reports from Sweden and the UK.[8,9] The proposed role of systemic ingestion of contact allergens as a cause of hand eczema is well known, although somewhat controversial.[11–16] It could explain the high prevalence of 'unexplained' contact allergy in our patients with hand eczema.

Hjorth *et al.*[7] proposed that irritant contact dermatitis is generally seen only in individuals who are constitutionally susceptible, and these individuals are not necessarily atopics. Endogenous/unclassifiable eczema was the commonest diagnosis in our patients with hand eczema and, together with irritant contact dermatitis, accounted for more than 75% of patients with hand eczema. There is no reliable test to differentiate irritant contact dermatitis from endogenous eczema. The high prevalence of endogenous/unclassifiable eczema could be due to our failure to recognize contact dermatitis in some of these patients. In 70% of our patients with irritant contact dermatitis of the hands the cause was occupational. Patients with this type of occupational dermatitis generally have a bad medical prognosis. Hjorth *et al.*[6] thought that they should be encouraged to continue their jobs as they still have a good social prognosis.

We have not studied the relationship between the pattern of hand eczema and its cause. Cronin[1] has observed in women that allergens, irritants or endogenous factors can produce similar and indistinguishable patterns of hand eczema. Elucidation of the cause of hand eczema still rests more on a detailed history and the results of patch testing than on the clinical distribution or pattern of hand eczema.

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Objective assessments of port wine stains: response to temperature change

S.W.LANIGAN AND J.A.COTTERILL

Department of Dermatology, The General Infirmary at Leeds, Great George Street, Leeds LS1 3EX, U.K.

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SUMMARY

Three objective measurements were made in 11 patients with facial port wine stains and in five controls. Laser Doppler flowmetry, reflectance spectrophotometry and skin surface temperature were all significantly elevated in the port wine stains compared with the normal contralateral cheeks and control skin ($P < 0{\cdot}05$).

Changes in skin blood flow and temperature after local heating and cooling of the skin were similar at all sites assessed and were not associated with significant changes in reflectance spectrophotometry, the parameter which correlated most closely with skin colour.

Port wine stains are congenital vascular naevi which consist of abnormally ectatic dermal blood vessels with an increased erythrocyte content. The naevus affects between 0·3–1% of the population.[1,2] The port wine stain is present at birth and persists throughout life, often darkening with the development of angiomatous nodules. As most port wine stains occur on the face, they may present considerable cosmetic disfigurement.

The colour of port wine stains correlates with the histological changes of vascular ectasia and erythrocyte content in the superficial dermis,[3] which increase with age resulting in a progression from a pink to a purple appearance. These changes are usually confined to the superficial 0·8 mm of the dermis.[3]

The aetiology of port wine stains has not been fully explained. The ectatic dilatation occurs in mature dermal blood vessels which stain normally with antibodies directed against vessel walls.[4] Smoller and Rosen[5] have demonstrated a reduction in the number of perivascular nerves in the naevi, and the distribution of port wine stains predominantly following sensory branches of nerves suggests a neurogenic aetiology with lack of neural modulation of vascular flow, which has been confirmed by Gaylarde *et al.*[6]

A wide variety of therapies have been attempted in the past to improve the appearance of port wine stains, mostly with disappointing results. However, with the use of argon,[7,8] dye,[9,10] and carbon dioxide[11] lasers, considerable improvement in the appearance of the port wine stain can be expected in 70–80% of cases.

Correspondence: Dr S.W.Lanigan

Most assessments of results have been made subjectively by direct observation and photography. The present investigation was performed to study various objective methods of assessing port wine stains and to investigate the responses of port wine stains to environmental stimuli in order to ascertain whether the ectatic vessels respond in a similar manner to normal skin.

METHODS

Subjects

Eleven patients were studied (eight males, three females, mean age 30 years, range 19–37 years). All patients had unilateral facial port wine stains, seven on the right, four on the left. Two port wine stains were predominantly pink-red, two were red, three red-purple and four predominantly purple in colour. No patient had received prior treatment of the areas assessed, and none had complications associated with port wine stains, for example glaucoma or Sturge-Weber syndrome.

The controls were five healthy volunteers (three males, two females, mean age 30 years, range 27–31 years).

Measurements

Three objective measurements were made on both cheeks of the patients and on the cheeks of controls. Skin surface temperature was measured using a thermocouple, cutaneous blood flow was measured using a laser Doppler flowmeter (Perimed, Sweden) and skin colour by reflectance spectrophotometry. The laser-Doppler flowmeter utilises the Doppler shift of helium-neon laser light which penetrates skin to a depth of 0·5–1 mm. The back-scattered Doppler shifted light reflected from blood is a function of the number and speed of movement of red cells in the area assessed and provides a reproducible and objective measurement of blood flow in the area in volts.[12,13]

The reflectance spectrophotometer is based on the theory that the logarithm of the inverse reflectance (LIR) of the skin approximates to the sum of absorbances of the skin's constituent pigments. The wavelengths of light used to illuminate the skin with this instrument (566 and 640 nm) provide information on both oxy- and deoxyhaemoglobin combined as a total blood content of the skin area assessed, and a reproducible objective measurement of this contribution to skin colour can be made.[14,15]

All measurements were recorded in a temperature controlled environment with an ambient temperature between 20 and 22°C, but not varying by more than 0·3°C during any individual observation. Subjects were recumbent and equilibrated in this environment for 30 min before measurements were made. They had not smoked, eaten or had a drink for 2 h prior to the studies, and no patient was taking medication.

Measurements were made over the zygomatic arch 6 cm lateral to the nasolabial fold. The laser Doppler probe was attached to the skin by double-sided tape; responses were recorded on a continuous paper read-out and continued until a stable baseline was achieved for 5 min at each site. Reflectance spectrophotometry measurements were performed on three occasions at each site, and the mean and standard error recorded. Skin surface temperature was recorded continuously until a stable temperature ±0·05°C was obtained for 5 min.

In the controls, initially all measurements were made on both cheeks, but as there was no difference between the two sides, in subsequent experiments either the left or the right cheek was assessed at random.

Responses to temperature change

The responses to external temperature change were recorded at the same sites. After a stable baseline was obtained, a glass container with a base 2 cm in diameter, full of ice, was applied to the malar skin for 2 min. After removal, laser Doppler flowmetry and skin temperature were measured simultaneously until baseline levels had been reached and maintained for 5 min; skin temperature measurements were recorded simultaneously on the contralateral cheek. The experiment was repeated with reflectance spectrophotometry measurements made on both cheeks, and with further laser Doppler flowmetry measurements on both cheeks and a visual observation of skin colour.

On a different day, the same measurements were performed after warming the skin by applying the same glass container full of water at 43°C for 2 min to the identical site.

Control experiments were performed with the empty container applied to the same site.

Statistical analysis

The results were compared using an analysis of variance applied to logged data.

RESULTS

The baseline assessments of the port wine stains, contralateral cheeks and control cheeks are shown in Table 1. Both reflectance spectrophotometry and laser Doppler flowmetry readings were significantly elevated in the port wine stain compared with normal contralateral skin and controls ($P < 0{\cdot}05$). There was no significant difference in skin temperature between the three groups, but the port wine stain was warmer than the contralateral skin in 10 of the 11 patients (mean difference 1·6°C, range 0–3·3°C). Reflectance and laser Doppler measurements did not show any consistent relationship with the colour of the port wine stain (Table 2), although the two patients with red port wine stains had higher recordings with both instruments.

On both cooling and heating of the skin, no changes were observed in any variable at the contralateral site.

TABLE 1. Baseline measurements in port wine stains, contralateral normal skin and controls. Values are means ± SEM

	Port wine stains	Contralateral skin	Controls
Reflectance (absolute units)	292·1 ± 38	146 ± 27	151 ± 25
Laser flowmetry (mV)	50·5 ± 18	28·6 ± 15	23·0 ± 6
Temperature °C	34·7 ± 1·3	33·2 ± 1·8	33·2 ± 1·1

TABLE 2. Baseline measurements in port wine stains in relation to colour. Values are means ± SEM

	Pink-Red ($n=2$)	Red ($n=2$)	Red-Purple ($n=3$)	Purple ($n=4$)
Reflectance (absolute units)	294·3 ± 12	338·0 ± 6	292·1 ± 14	291·7 ± 16
Laser flowmetry (mV)	41·0 ± 5	52·5 ± 15	45·2 ± 7	48·2 ± 3

Changes in laser Doppler flowmetry and skin surface temperature after cooling are shown in Table 3.

The maximal fall in blood flow and skin temperature was found in the first measurements 30 s after removal of the glass container, and time to recovery was measured as time to reach baseline after initial application of the container.

There were no significant differences in the magnitude of change induced, nor in time to recovery of skin blood flow and skin temperature, at the three sites after cooling the skin. In each patient the return to baseline of blood flow and skin temperature occurred in parallel.

There were no significant changes in reflectance spectrophotometry readings nor any noticeable colour change at any of the sites tested.

Changes in laser Doppler flowmetry readings and skin surface temperature after heating are shown in Table 4. There were no significant differences between the changes in the three groups. The return to baseline of skin temperature paralleled that of skin blood flow.

TABLE 3. Changes in laser Doppler flowmetry results and skin temperature on cooling the skin. Values are means ± SEM

	Port wine stain	Contralateral skin	Controls
Decrease in laser Doppler flowmetry reading (fold)	1·6 ± 0·5	0·7 ± 0·6	1·9 ± 0·7
Time to recovery (min)	10·6 ± 3·5	8·4 ± 5·5	11·5 ± 6·9
Temperature fall (°C)	5·4 ± 3·2	5·9 ± 3·7	5·3 ± 4·8
Time to recovery (min)	11·7 ± 2·8	11·8 ± 5·3	10·0 ± 1·2

TABLE 4. Changes in laser Doppler flowmetry results and skin temperature on heating the skin. Values are means ± SEM

	Port wine stain	Contralateral skin	Control
Increase in laser Doppler flowmetry reading (fold)	1·5 ± 0·4	2·3 ± 1·9	2·0 ± 0·6
Time to recovery (min)	13 ± 1·4	9·7 ± 2·5	15 ± 0·5
Temperature rise (°C)	1·7 ± 1·4	2·1 ± 1·8	1·5 ± 1·1
Time to recovery (min)	10·0 ± 3·9	11·2 ± 2·6	15·0 ± 0·5

No changes in skin colour or in reflectance spectrophotometry readings were observed in the port wine stain, or in the contralateral cheek of patients on heating the skin. In one control only, erythema was observed with an increase in reflectance spectrophotometry readings.

DISCUSSION

With advances in the treatment of port wine stains by lasers it is becoming increasingly important to develop more objective assessments than clinical observation to assist in predicting the outcome of therapy, quantification of results and understanding the pathophysiology of the naevi.

Most objective assessments of treated port wine stains have been by histological changes,[16–18] but in a treatment designed to improve cosmetic appearances, non-invasive methods of assessment are desirable. It has been demonstrated that the colour of port wine stains correlates with erythrocyte filling of ectatic blood vessels,[3] and that darker port wine stains with a higher percentage filling of blood vessels have a more favourable response to therapy.[19]

Non-invasive methods of assessing port wine stains have been based on the abnormal vasculature of the naevi and have included thermography,[20] transcutaneous microscopy,[21] reflectance spectrophotometry,[22,23] and laser Doppler flowmetry.[23] Methods which detected a large difference between the port wine stain and normal skin correctly predicted a favourable outcome to laser therapy.

The aims of the present study were to investigate objective methods of assessment of port wine stains which were reproducible and correlated with the clinical appearance of the naevus, and to use these methods to investigate the responses of port wine stains to local temperature change. The responses of port wine stains to cooling and warming were studied because of the possibility of a neurogenic pathogenesis[5] and to consider whether manipulating port wine stains may offer theoretical advantages in laser therapy. In addition, because of the occasional occurrence of naevus anaemicus-like lesions in association with port wine stains,[24] it was of interest to determine whether normal-appearing skin in patients with port wine stains reacted in a similar manner to controls.

All three methods studied detected a significant difference beteen port wine stains and normal skin, which was reproducible.

The responses of port wine stains to local temperature changes were similar to those of contralateral normal skin and facial skin of controls. By cooling the skin, it was possible to induce changes in skin blood flow and in skin temperature to levels similar to those in normal skin, without changes in skin colour either visible or detectable by reflectance spectrophotometry.

Similarly, by warming the skin, substantial increases in laser Doppler flowmetry and skin temperature were not associated with changes in skin colour. Thus, from these studies it can be seen that reflectance spectrophotometry correlated best with clinical appearance.

The absence of changes in reflectance spectrophotometry may be due to the relative insensitivity of this instrument, but it has been demonstrated to be more sensitive than the human eye in detecting skin colour changes.[25] The persistence of skin colour after profound changes in laser Doppler flowmetry suggests a number of possibilities. Laser Doppler flowmetry has been shown to be more sensitive than the human eye in detecting erythema[12] and it may be that changes induced in these experiments were inadequate to produce sufficient vasodilatation or vasoconstriction to induce skin colour change. However, the demonstration of laser Doppler flowmetry readings in cooled port wine stains below those in normal contralateral skin, without colour changes suggests that this cannot be the only explanation.

Although most of the ectatic vessels in port wine stains occur in the first 1 mm of skin, vessels below this may be contributing to skin colour, and changes in them would not be detectable by laser Doppler flowmetry. However, this should be a minor effect in comparison with the large vascular bed in the superficial dermis.

Finally, as blood flow is altered without change in colour, this suggests that the erythrocyte content of blood vessels remains constant, and any vasoconstriction induced by cooling would be associated with an increased percentage vascular filling. The increased erythrocyte filling of blood vessels in port wine stains after cooling the skin has been demonstrated histologically.[26] Similarly, warming the skin whilst increasing blood flow may decrease vascular filling.

There are theoretical advantages in increasing the erythrocyte content of vessels in port wine stains in laser therapy, and cooling of port wine stains with ice has resulted in improved results,[26,27] which are probably due to alteration of the erythrocyte content of the vessels in addition to epidermal cooling.

The poor correlation between baseline measurements of reflectance and laser Doppler flow and colour of port wine stains between patients is not surprising. Reflectance spectrophotometry measures combined oxygenated (red) and deoxygenated (purple) haemoglobin as a contribution to skin colour, whilst laser Doppler flowmetry measures number and movement of red blood cells and is dependent on flow as well as haemoglobin content. These measurements are of more value in following sequential changes in individual patients, for example, after laser therapy.[28]

The simultaneous measurement of skin colour and blood flow provides a useful model for investigating potential adjuncts to laser therapy.

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Treatment of mycosis fungoides with recombinant interferon-α2a^2 alone and in combination with etretinate

K.THESTRUP-PEDERSEN*, R.HAMMER†, K.KALTOFT‡,
H.SØGAARD§ AND H.ZACHARIAE*

*The Department of Dermatology, Marselisborg Hospital, University of Aarhus, †The Department of Clinical Research, Roche A/S, Copenhagen, ‡Institute of Human Genetics, University of Aarhus and §The Department of Pathology, Kommunehospitalet, Denmark

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SUMMARY

Eleven patients with mycosis fungoides (MF) were treated with recombinant alpha-interferon (rIFN-α2a^2) in combination with etretinate (seven patients) or alone. One patient, who also received etretinate, went into complete remission and remained without signs of MF after 18 months. Six patients experienced partial remission; one of these was treated with rIFN-α2a alone and was clinically in complete remission, but had still a pleomorphic skin infiltrate. Two patients were non-evaluable, and two stopped therapy due to progressive disease. Five patients discontinued therapy due to side-effects although three had partial remission of their disease. Only four patients received 12 months therapy. The study shows that rIFN-α2a in combination with etretinate or alone can induce remission of MF.

The pathogenesis of mycosis fungoides (MF) remains unsolved although a viral aetiology is suspected.[1–4] The disease carries a high mortality rate in advanced cases.[5–7] A wide variety of therapeutic approaches have been successful in obtaining prolonged remissions[8–13] including etretinate [14,15] and 13-cis-retinoic acid,[16] but new potentially curative therapeutic modalities are still needed.

Interferon has recently been used systemically with some success in advanced cases of cutaneous T cell lymphoma, refractory to two or more standard therapeutic regimens.[17,18] It has also been given locally in the early stages of the disease.[19] These observations and the possible viral aetiology of MF have created an interest in the use of interferon in this disease. We present here the results of treatment of 11 patients with MF, with recombinant interferon α2a^2 (rIFN-α2a^2, Roferon-A®) alone or in combination with etretinate.

Correspondence: Dr K.Thestrup-Pedersen, Marselisborg Hospital, DK8000 Åarhus C., Denmark.

METHODS

The study was approved by the Ethical Committee for the County of Aarhus. All patients gave informed consent.

Patients

Eleven patients (three women and eight men) with clinically active MF, were included in the study. One patient (Patient 10) was included twice; he was treated first with rIFN-α2a[2] in combination with etretinate for a 12-month period and then treated for 7 months with rIFN-α2a[2] alone. Clinical details are given in Table 1.

Treatment

The treatment schedule consisted of 3 million units of rIFN-α2a given subcutaneously for 3 days, and then increased every 4th day to 9, 18, and 36 million units daily. After 3 months rIFN-α2a[2] was given three times weekly up to 12 months; one patient continued treatment for 18 months. The dosage of rIFN-α2a[2] was adjusted according to the side-effects, which were treated with paracetamol.

Etretinate (Tigason®) (0·7 mg/kg) was given daily with the rIFN-α2a[2] to Patients 1, 4, 6, 8, 9, 10 and 11, but was not given to the remaining patients in order to evaluate the efficacy of rIFN-α2a[2] alone. Due to side-effects we reduced the maximal daily dosage of rIFN-α2a[2] from 36 million to 18 million units.

TABLE 1. Clinical details of patients included in the trial

Patient no.	Sex	Age (years)	MF stage*	Disease duration (years)	Previous treatment
1	M	50	II	2	None
2	M	67	II	3	PUVA
3	M	67	II	2	None
4	F	60	II	10	Mustine, PUVA, transfer factor
5	F	70	II	2	None
6	M	59	IVa	1½	Mustine, X-rays
7	F	48	IVa	6	Steroids
8	M	49	IVa**	6	Mustine, steroids
9	M	66	IVa**	20	Mustine, steroids
10‡	M	49	IVb	5	Mustine, chemotherapy
10	M	50	IVb	6	Mustine, rIFN-α2a
11	M	43	IVb	13	Mustine, chemotherapy

* Staging according to the Scandinavian Mycosis Fungoides Study Group.[27]

** Erythroderma, but no leukocytosis or Sézary cells in peripheral blood.

‡ Patient 10 was given interferon twice. During the first part of the study etretinate was also administered.

Investigations

Clinical examinations and laboratory investigations were performed every second week for 2 months, then monthly. Screening for rIFN-α2a^2 antibodies was performed monthly. Serum was screened for reactivity against the cell line Se-Ax, a retrovirus releasing cell line from a patient with Sézary's syndrome[20] (Kaltoft *et al.*, unpublished). An anti-Se-Ax antibody was found in Patient 1, who went into complete remission. Staining of the Se-Ax cell line is a granular, cytoplasmic staining, which apparently is directed against an antigen induced by α-interferon itself, because the continuous cell lines HUT 78 and K-562 do not contain a similar antigen, except after incubation with 10 U/ml rIFN-α2a^2. We did not detect antibody in 100 normal sera, or in sera from other patients with MF or Hodgkin's lymphoma.

Evaluation of effectiveness of therapy

The evaluation of the therapy was based upon the size of the affected areas, redness, scaling, infiltration, and itching. Each parameter was evaluated separately for the head, neck, trunk and extremities. The patients were considered to be in complete remission if all clinical and histological signs of MF had disappeared, and to be in partial remission if the disease activity had diminished clinically and histologically. Patients were considered non-evaluable if the treatment was stopped within 1 month due to side-effects, and were considered to have progressive disease if there were signs of increased disease activity.

RESULTS

Clinical response

The results are shown in Tables 2 and 3. One patient (Patient 1) was brought into complete remission with rIFN-α2a^2 and etretinate and, at the time of writing had remained in remission for 18 months without further treatment. Six patients, of whom two received therapy for 12 months, experienced partial remission. One of these patients (Patient 2) completed 12 months therapy with interferon alone and was clinically in complete remission (Figs 1a and b and 2a and b), but histologically there was still a pleomorphic infiltrate, although this was not diagnostic for MF (Figs 3a and b). He was, therefore, given X-irradiation on three previous plaque and tumour sites because of these changes and kept on rIFN-α2a^2 for a further 6 months, which brought him into complete remission. Two patients were not evaluable after a 1-month treatment period, when they stopped due to side-effects, and three had to stop due to progressive disease—one of them had previously experienced a partial remission following rIFN-α2a^2 and etretinate (Patient 10).

Tolerance and side-effects

Only one patient (Patient 10) tolerated the maximal dosage of 36 million units. The others were maintained on a dosage ranging from 3 to 9 million units per injection. Every patient experienced flu-like symptoms and fever, and three patients withdrew because of this. Eight patients had leukopenia at some stage, four lost over 3 kg in weight, and four developed pneumonia (sudden fever with chills and unilateral lung crepitation); all responded to antibiotics. One patient developed severe depression and another severe purpura on the feet without accompanying thrombocytopenia. The symptoms disappeared following discontinuation of therapy. One patient (Patient 4) experienced a pronounced hearing loss necessitating the use of hearing aids. Discontinuation of therapy lead to the return of normal hearing within 3

TABLE 2. Details of treatment and outcome

Patient no.	Disease stage	Etretinate	Duration of treatment (months)	Condition at end of treatment	Length of follow-up (months)	Condition at end of follow-up	Other therapy
1	II	+	12	CR	18	CR	—
2	II	...	12	PR	4	CR	X-rays
3	II	...	1	PD	12	CR	Chemotherapy[15]
4	II	+	2#	PR	24	PR	Chemotherapy, mustine
5	II	...	2#	PR	12	NC	Topical steroid
6	IVa	+	2#	PR	11	PD**	—
7	IVa	...	12	PR	3	PD	—
8	IVa	+	1#	NE	15	PR‡	—
9	IVa	+	1#	NE	14	PR	Systemic steroid
10	IVb	+	12	PR	7	PD	Mustine
10	IVb	...	5	PD	12	PD	Chemotherapy, mustine
11	IVb	+	2	PD		PD**	

# withdrawn due to side effects.
** Died of MF.
‡ Died of cirrhotic hepatitis.
CR: complete remission.
PR: partial remission.
PD: progressive disease.
NE: non-evaluable.

TABLE 3. Clinical response to rIFN-α2a[2] therapy at the end of treatment in combination with etretinate or alone

Treatment	No. of patients			
	Complete remission	Partial remission	Progressive disease	Non-evaluable
rIFN-α2a + etretinate	1	3	1	2
rIFN-α2a	0	3*	2	0

*Patient 2 came into complete remission after X-ray and continued rIFN-α2a[2] therapy.

months. Another patient (Patient 10) developed symptomless retinal exudates. The changes disappeared spontaneously without discontinuation of therapy.

Histology

Histological examinations were performed in patients showing improvement of their disease. There was a diminution of the pleomorphic infiltrate, and Pautrier's microabcesses disap-

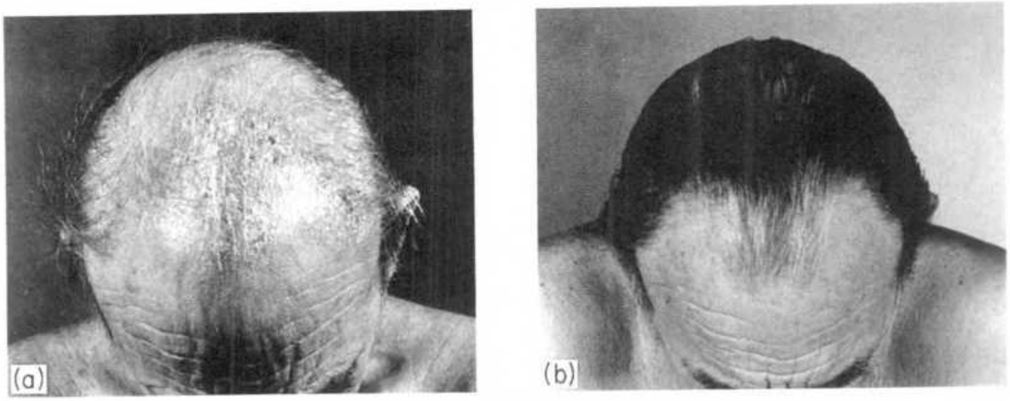

FIGURE 1. Scalp lesion in Patient 2, (a) before and (b) after 9 months of therapy.

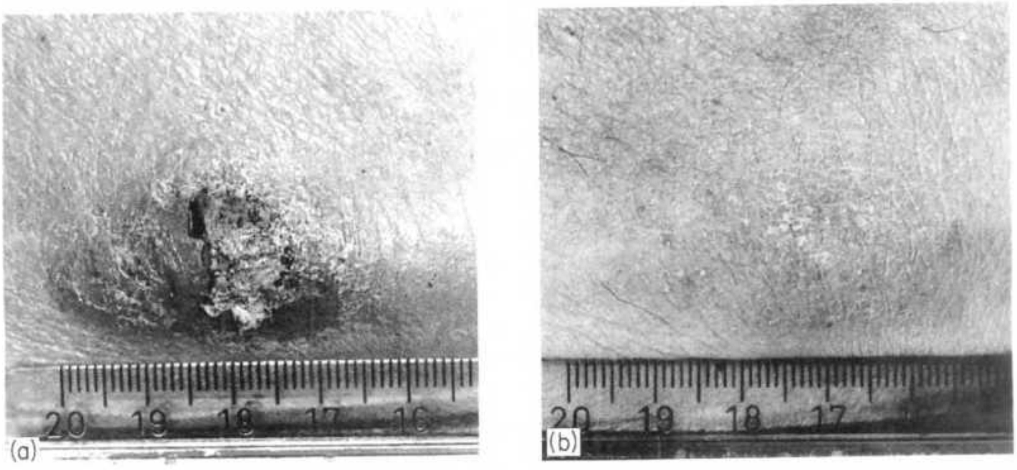

FIGURE 2. Patient 2. A plaque lesion on the left lower arm (a) before and (b) after 9 months of therapy.

peared. However, it was evident that some patients in spite of clinical improvement still had abnormal pleomorphic infiltration in the affected area (see Figs 3a and b).

Serological effects

We found no anti-rIFN-$\alpha 2a^2$ antibodies. One patient (Patient 1) out of four investigated showed a temporary increase in serum gamma globulin during rIFN-$\alpha 2a^2$ therapy from 10·8 g/l to 20·0 g/l. He also developed an IgG antibody against the Se-Ax cell line, which was not present prior to therapy, but present at a titre of 1:20 following 5 months of therapy and 1:100 at the end of treatment. The antibody disappeared after stopping therapy.

ILLUSTRATIVE CASE HISTORIES

Patient 10

This patient, a 49-year-old man, developed MF in 1980 with lymph node involvement (stage IVb). Chemotherapy brought him into remission. A relapse in 1984 was treated with rIFN-

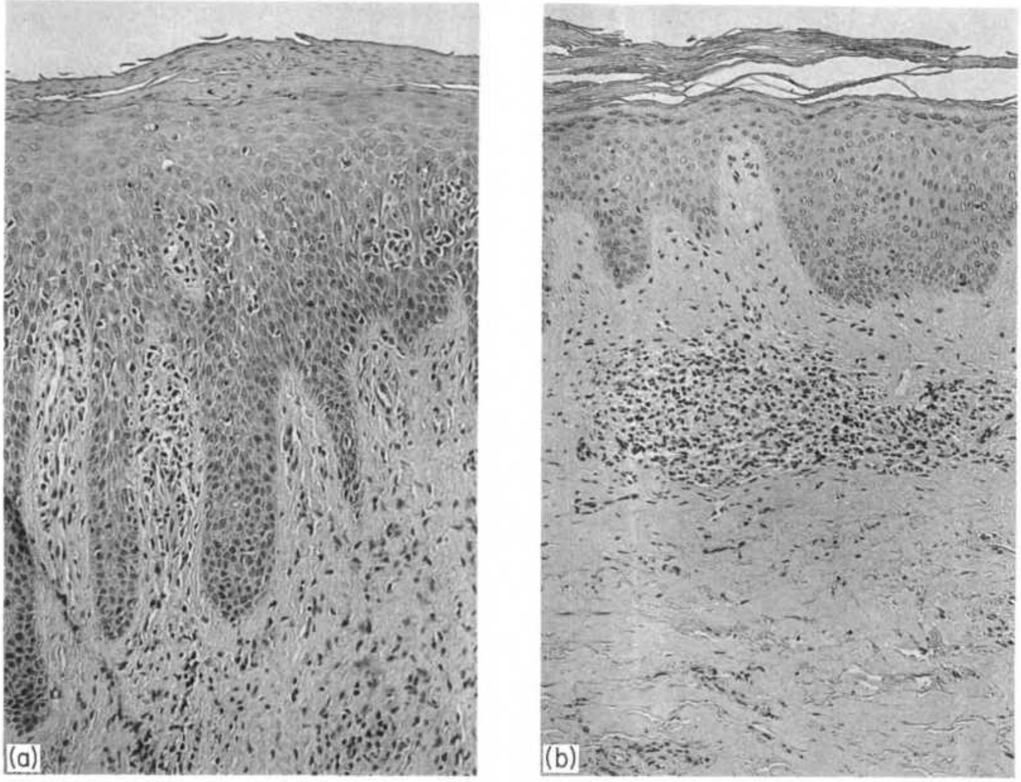

FIGURE 3. Patient 2. Infiltrate in the plaque on the left lower arm (a) before and (b) after 9 months of therapy. Note that there is still a lymphocytic infiltrate in Figure 3b even though the clinical appearance is normal (Fig. 2b). The exocytosis has disappeared.

$\alpha 2a^2$, 36 million units daily and etretinate bringing him into partial remission. Lesions on the head, trunk and arms disappeared completely, but new lesions developed on the right lower leg. He then received topical nitrogen mustard every fortnight for 7 months, when a new relapse occurred. He then received rIFN-$\alpha 2a^2$ therapy alone and obtained a temporary partial remission, but could only tolerate 9 million units per dose. After 5 months of treatment, his MF progressed and nitrogen mustard and later chemotherapy were re-instituted bringing him into complete remission desite the fact that he had had the disease (stage IVb) for 7 years.

Patient 7

This patient had stage IVa MF, which went into partial remission during rIFN-$\alpha 2a^2$ therapy alone. During 12 months of therapy she was without new skin tumours. Two months after stopping therapy she developed a 2 × 2 cm infiltrated tumour on the right upper arm.

DISCUSSION

rIFN-$\alpha 2a^2$ has been used in the treatment of MF[17-19] and other lymphomatous disorders.[21,22] A previous study on 20 patients with advanced cutaneous T cell lymphomas[17,18] showed a clinical response lasting more than 3 months in nine patients, but side-effects were a major problem. Local injection of rIFN-$\alpha 2a^2$ has also been effective in the early stages of the disease.[19]

The present study has shown that rIFN-$\alpha 2a^2$ may have a role in the treatment of MF. The most beneficial effects were seen in patients with early disease. The best responses (complete or partial remission) were found in two patients in whom rIFN-$\alpha 2a^2$ was the first specific treatment for MF. The patient who went into complete remission did also receive etretinate, and Patient 10 was able to remain longer on rIFN-$\alpha 2a^2$, when combined with etretinate. rIFN-$\alpha 2a^2$ may also delay the need for chemotherapy.

The apparent discrepancy between clinical improvement and the continued presence of a pleomorphic infiltrate on histological examination has been observed by others.[17] It implies that interferon should be used for longer periods than 12 months.

The side-effects were pronounced in some patients, and were similar to those previously observed,[17,18,22] although the side-effects of temporary hearing loss and retinal exudates have not been described before to our knowledge. When severe side-effects occurred we only stopped treatment for a few days. It is possible that longer intervals might have prevented five patients stopping treatment due to side-effects.

Immunoglobulin (Ig) is increased in the serum of some patients with MF[23]. This is apparently in contrast to decreased Ig-release *in vitro* by lymphocytes from MF patients.[24] B lymphocytes *in vitro* release less Ig after addition of α-interferon.[25] We observed a marked rise in Ig during rIFN-$\alpha 2a^2$ therapy in one of four patients, concomitant with his clinical improvement.

The development of an IgG antibody probably reacting with a proliferation antigen has not been described before to our knowledge. The antibody was not a T lymphocytotoxic antibody, which has been observed by others to be present in MF.[26] Further characterization of the cellular antigen is needed.

We see rIFN-$\alpha 2a^2$ as a promising new therapeutic modality in MF. rIFN-$\alpha 2a^2$ is convenient to adminster and may also be useful where age or physical disability make a patient unsuitable for other forms of treatment.

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Metastatic malignant clear cell hidradenoma associated with bullous pemphigoid

ALEXANDRA KAZAKIS, C.WOOD AND REGINA ANDERSON

Division of Dermatology, Department of Medicine, University of Maryland School of Medicine,
22 South Greene Street, Baltimore MD 21201, U.S.A

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SUMMARY

The clinicopathological features of a case of metastatic malignant clear cell hidradenoma (MCCH) occurring in a 72-year-old black male are reported. Unusual features of the case included the location of the tumour on the posterior trunk, the probable origination of the tumour from a benign appendageal tumour, and the occurrence of a bullous eruption immediately after surgical removal of the tumour. Clinicopathological aspects of MCCH are reviewed and treatment modalities, including chemotherapy, are discussed.

Malignant clear cell hidradenoma (MCCH) is a rare form of eccrine carcinoma. In a recent study, the incidence of this tumour was reported to be 6% of eccrine gland carcinomas which were in turn detected in one of every 13,000 routine dermatological biopsies.[1] Many isolated cases of MCCH have been reported, and recently a 10-year review of nodular hidradenomas and hidradenocarcinomas has been published.[2]

We report here a case of malignant clear cell hidradenoma with some unusual clinical features.

CASE REPORT

A 72-year-old black man had noticed an asymptomatic lesion on his right buttock which had been progressively enlarging for at least 10 years. Approximately 2 weeks prior to presentation, the area involved was traumatized resulting in pain, bleeding, and enlargement of the lesion. The patient was otherwise healthy.

On physical examination, a 5 cm diameter, flesh coloured, nodular mass surrounded by an erythematous indurated patch was noted on the right buttock (Fig. 1). The mass exuded a straw coloured fluid through a superficial ulceration and was extremely tender. A 2 × 2 cm firm, non-tender lymph node was palpated in the right inguinal area.

The patient was treated for 7 days with erythromycin (1 g per day) for suspected cellulitis, and

Correspondence: Alexandra M.Kazakis M.D.

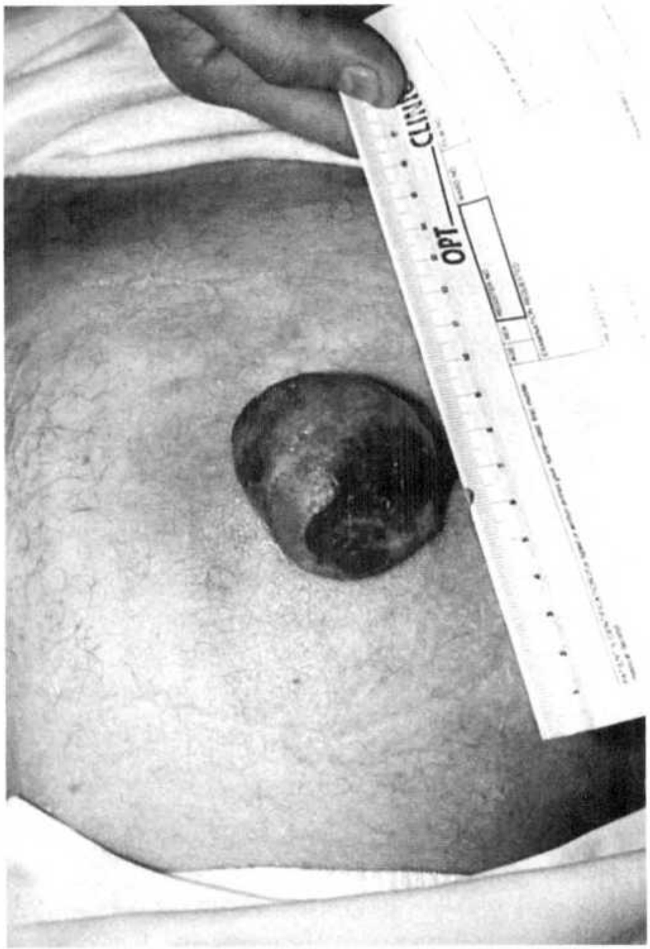

FIGURE 1. Appearance of the lesion at initial presentation.

then underwent an extensive debulking procedure of the nodular mass. The specimen was transected and appeared to contain multiple irregular cystic cavities and fibrotic nodules.

The excision site exhibited regrowth of the mass and several satellite lesions 3 weeks after surgery. The patient was referred for wide surgical excision of the involved area. He refused biopsy of his enlarged right inguinal node. Laboratory studies including SMA-12, full blood count, liver function tests, bone scan, and chest X-ray were all normal.

One week after wide excision, a blistering eruption consisting of multiple tense vesicles and bullae on erythematous bases and many crusted erosions appeared in the right groin and right inner thigh (Fig. 2). A biopsy of one of the vesicles was consistent with bullous pemphigoid. Direct immunofluorescence studies on perilesional skin failed to show any staining.

Topical treatment of the eruption with fluocinonide cream controlled the localized eruption for 3 months. After this time, new vesicles appeared on the trunk and extremities, as well as the inguinal area, and the patient was commenced on 60 mg oral prednisone per day. Because the eruption persisted, the prednisone was increased to 100 mg per day. The eruption began to

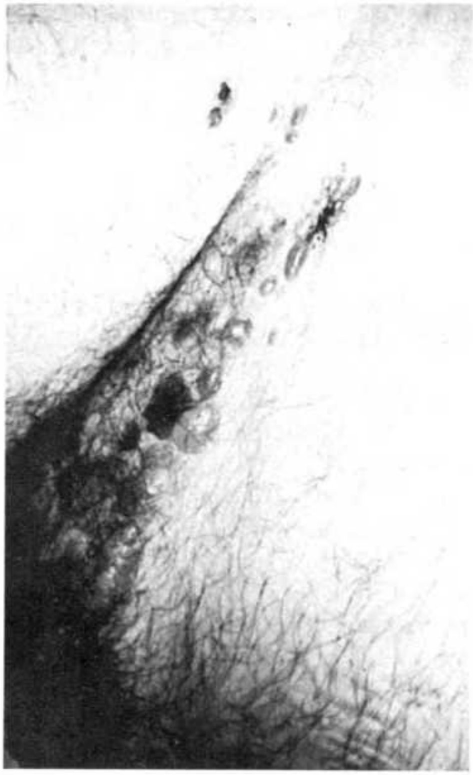

FIGURE 2. Vesiculobullous eruption in the right groin.

resolve and the prednisone was then slowly tapered off. Five months after resection of the tumour, while on 40 mg prednisone per day, the patient noted swelling of his right leg and scrotum. A firm inguinal mass was palpated in the right inguinal area. Again, the patient refused biopsy and haematological studies.

One month later, the patient's bullous disease flared with numerous bullae and vesicles appearing on his trunk, extremities, and mucous membranes and he was restarted on 60 mg oral prednisone per day. One week later he was hospitalized with erosions, vesicles and bullae on his trunk, extremities, mucous membranes, palms and soles, massive oedema of his right leg and scrotum, violaceous nodules on his abdomen, and a 14 × 5 cm firm non-mobile right inguinal mass. He was treated with high daily doses of prednisone (200 mg) and commenced on amikacin for a *Pseudomonas* urinary tract infection. Indirect immunofluorescence for basement membrane zone antibody was negative. Bullae formation ceased, and re-epithelialization ensued. However, the patient developed progressive renal failure thought to be secondary to aminoglycoside toxicity. He died 6 weeks after admission.

Autopsy showed massive tumour growth in the skin and subcutaneous tissue of the back and metastases to periaortic, hilar, supraclavicular, right inguinal and pelvic lymph nodes with direct tumour ulceration into the inferior vena cava, as well as widespread metastases to liver, spleen, lung, pericardium and bone.

HISTOLOGY

Histological examination of the excised tissue obtained at the first operation showed three cysts, 4–12 mm in diameter in the dermis and subcutis adjacent to a solid tumour, 2 cm wide by 1 cm thick, which extended to the deep margins of the specimen. The cysts were lined by a layer of cuboidal, flattened or polygonal epithelial cells, from one to many layers thick, and contained eosinophilic colloid-like secretory material and scattered foamy macrophages. The epithelium overlaid a thick basement membrane, and showed focal epithelial cellular atypia with large nuclei (Fig. 3). These cells were continuous focally with the extracystic solid tumour. The solid tumour consisted of closely packed large and small irregular islands of large polygonal cells with eosinophilic or vacuolated clear cytoplasm and large round or irregularly shaped pale staining nuclei. Numerous mitoses and a scanty vascular stroma were seen. PAS staining with and without diastase digestion showed abundant cytoplasmic glycogen in the extensive clear celled areas of solid tumour. Immunoperoxidase staining showed abundant tumour cell cytoplasmic staining for cytokeratin and carcinoembryonic antigen (CEA), especially in the clear cells (Fig. 4). The tumour extended to the lateral margins of the specimen. Examination of the tissue removed at the second operation revealed similar changes except that normal tissue bordered the excision margins.

Autopsy specimens of tumour from the skin of the back and groin, and from the lungs, liver and spleen showed essentially similar tumour tissue, composed of small irregular islands of tumour cells separated by narrow bands of poorly cellular fibrous tissue. The tumour cells were large and polygonal, with abundant clear, or eosinophilic, often peripherally condensed cytoplasm. The nuclei were large and irregular, often with large nuceoli. Mitoses were numerous. In the lungs, tumour cells were seen both in nodular aggregates and in small blood vessels (Fig. 5).

A biopsy from a vesicle in the groin showed a sharply demarcated subepidermal vesicle. The epidermis in the roof of the blister was intact and showed no necrosis. The vesicle contained fibrin and numerous inflammatory cells, nearly all of which were eosinophils. Red cells were also present. There was a superficial perivascular and diffuse upper dermal infiltration of eosinophils and scanty lymphocytes. Epidermal regeneration was seen at both lateral margins of the vesicle. The changes were diagnostic of bullous pemphigoid (Fig. 6).

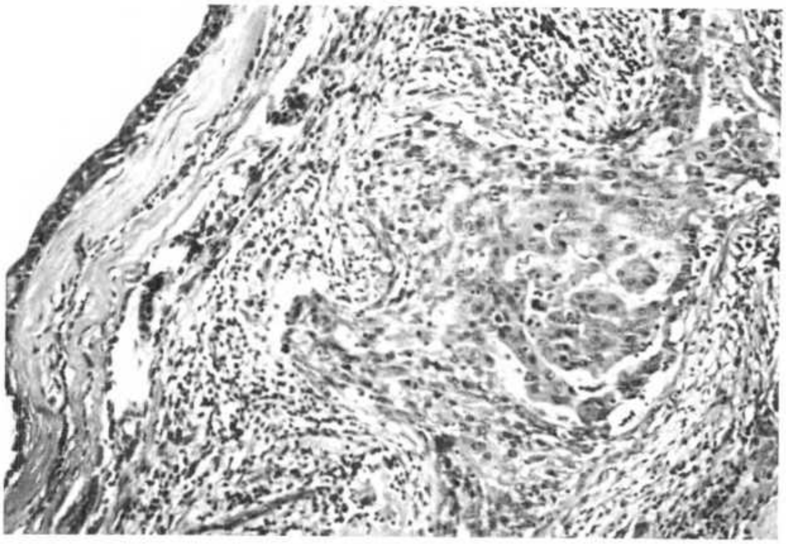

FIGURE 3. Cyst with atypical lining epithelium and adjacent solid tumour.

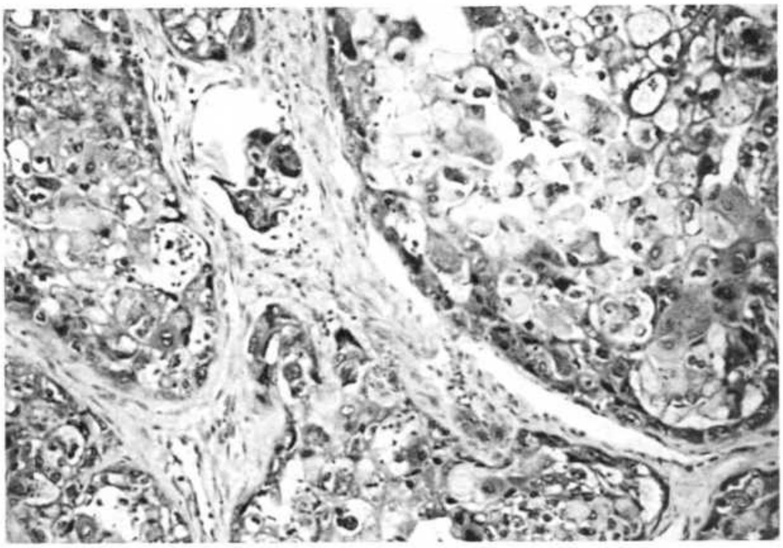

FIGURE 4. Immunoperoxidase stain showing abundant cytokeratin (black staining) in cytoplasm of tumour cells.

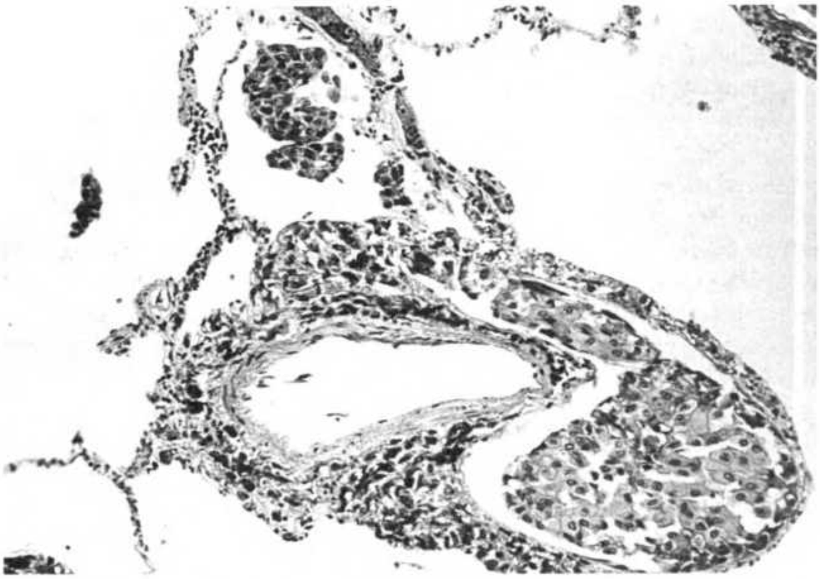

FIGURE 5. Metastatic clear cell hidradenoma in small pulmonary vessels at autopsy.

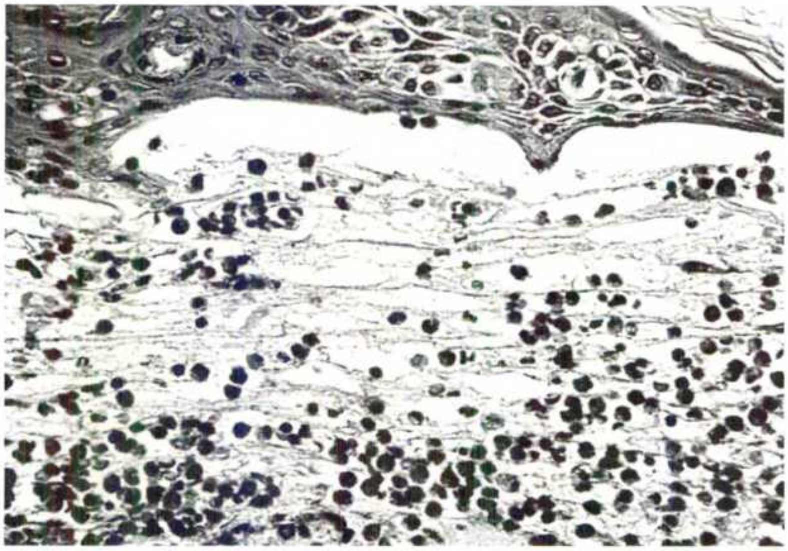

FIGURE 6. An intact subepidermal vesicle containing fibrin and inflammatory cells, predominantly eosinophils. A superficial perivascular and diffuse upper dermal infiltration of eosinophils and scanty lymphocytes is also present.

DISCUSSION

Our case illustrates many of the characteristics of malignant clear cell adenocarcinomas, but also presents some unusual features.

As with many malignant clear cell hidradenomas, our patient's lesion had no clinically distinguishing aspects. MCCH clinically can resemble a variety of entities including haemangiomas, lymphangiomas, or even dermatofibrosarcoma protuberans.[3,4] The tumour usually appears as a solitary, slow-growing asymptomatic, flesh coloured papule or nodule,[5,6] most commonly 0·5 cm to 3 cm in size,[6] although lesions as large as 12 cm have been reported.[7,8] The diagnosis can only be made histologically.

Adenocarcinomas of eccrine sweat glands show many microscopic variants. In a recent study of 35 cases, eccrine porocarcinoma and syringoid eccrine carcinoma accounted for 30 cases, and mucinous eccrine carcinoma and clear cell carcinoma only three and two cases, respectively.[1] Both clear cell tumours were confined to the dermis. In our patient, the finding of cutaneous epithelial cysts, with a predominantly benign, but focally atypical, epithelial lining, continuous at points with a deeply infiltrating malignant epithelial tumour, is consistent with a carcinoma arising from a benign cystic skin appendage tumour. The residual cystic component of the tumour did not show the features of an apocrine hidrocystoma or syringocystadenoma papilliferum, and possibly represented an eccrine hidrocystoma. The solid invasive tumour, because of the clear cells with abundant glycogen and positive cytokeratin and CEA staining, was more definitely classified as a malignant clear cell acrospiroma (hidradenoma). Positive staining for CEA in glandular tumours, primary in the skin, is good evidence of sweat gland differentiation[9] and has been reported in sweat gland carcinomas.[10] A mouse monoclonal anti-human cytokeratin (Dako-CK1) was used, which stains keratin 18 (molecular weight 45 kD) and keratin 6 (56 kD). This has been shown to identify tumour cells of epithelial or, less commonly, mesothelial origin.[11,12]

MCCH has been reported to occur in various sites anteriorly, most commonly the face, scalp, and anterior trunk.[6] A posterior location, although reported,[4] is unusual. The occurrence of our patient's tumour on the right buttock re-emphasizes the fact that these tumours can occur anywhere on the body.

The age of our patient, 72 years, was typical as this tumour most often appears in the elderly, although it has been reported to occur in neonates and children.[2,4] Although most of these tumours remain localized for many years, they usually are regarded as malignant from the beginning.[13] In contrast, our patient's tumour appears to have arisen from a benign appendageal tumour. While spontaneous regression can occur,[2] several authors have reported widespread metastases to bone and lymph nodes.[4,14] Our patient was in the latter category with metastases to bone, lymph nodes, liver, lung and pericardium.

Since MCCH is rare and can vary from an isolated localized malignancy to an aggressive metastatic process, controversy still exists over the best means of adequately treating this tumour. Most authors advocate wide local excision of the lesion.[4,5,15] The importance of complete excision is stressed because of the propensity of this tumour for rapid regrowth following trauma or inadequate excision.[5,7,16] This was illustrated by our patient's report of rapid growth of his tumour following trauma and the rapid regrowth following the debulking procedure. Lymph node dissection has been recommended for large lesions.[5,15] Our experience supports early lymph node biopsy and dissection. Prophylactic lymph node excision in cases of large tumour load should be considered in light of this tumour's very aggressive course.

Because these tumours are radioresistant and some authors believe that X-ray treatment may actually accelerate growth, radiotherapy is contraindicated.[5,16] Recently, chemotherapy has been employed in five cases of MCCH in children.[4] In the three children without evidence of tumour post excision, two remained disease free 2 years after chemotherapy, the other died of metastatic disease. No effect was seen with chemotherapy in those with disseminated disease. Our patient was not offered chemotherapy, as it frequently warrants many blood transfusions and the patient refused all blood products because of his religious affiliation.

The most unusual aspect of this case was the occurrence of a localized vesiculobullous eruption after surgical excision. Histologically, this was entirely consistent with bullous pemphigoid. The negative immunofluorescence was not surprising because the vesicle biopsied was located on the left thigh, a site which has been reported to give a low incidence of positive immunofluorescence in bullous pemphigoid.[17] An association between bullous pemphigoid and malignancy is disputed. Hodge *et al*[18] found eight concurrent malignancies in 35 patients with sero-negative bullous pemphigoid (23%) in contrast to only four malignancies in 89 patients with bullous pemphigoid with positive indirect immunofluorescence for basement membrane zone antibodies (4%). In our patient, indirect immunofluorescence was negative; however, at the time this test was done, he was in hospital and on high doses of steroids. It was interesting that a severe exacerbation of the vesicobullous eruption occurred with exacerbation of his malignancy. It is possible that the patient's cutaneous eruption was a result of an alteration in his immune status induced by the removal of the bulk of his tumour mass, and his later exacerbations were associated with alteration in his immune status caused by large increases in his tumour mass. To our knowledge bullous pemphigoid has never been reported previously in association with MCCH.

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Recurrent pseudo-scarlatina and allergy to pseudoephedrine hydrochloride

B.J.TAYLOR AND M.B.DUFFILL

Department of Dermatology, Waikato Hospital, Hamilton, New Zealand

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SUMMARY

A case of recurrent pseudo-scarlatina is described. Challenge with pseudoephedrine hydrochloride reproduced a typical attack but pseudoephedrine ingestion could not be related definitely to all episodes. Allergy to pseudoephedrine or codeine should be considered as a cause for recurrent scarlet fever-like eruptions.

CASE REPORT

A 32 year-old housewife was first seen in April 1985 with an acute scarlet fever-like illness. There was a history of five similar episodes over the previous 12 years. Her general practitioner had been unable to find a cause for the attacks. An original diagnosis of scarlet fever had been discarded because of lack of significant associated pharyngeal symptoms, negative throat cultures, normal streptococcal antibodies and the recurrent nature of the attacks. However, the attacks appeared to respond to penicillin therapy.

The latest attack had begun with feelings of tiredness and irritability and a mild sore throat. The patient then developed a prickly uncomfortable feeling under her underwear followed by erythema under the breasts, in the axillae and groins, on the sacrum and behind the knees. Her entire skin was hypersensitive. Hot and cold sensations developed with pronounced malaise. The erythema then became generalized. She was febrile with rigors. Examination showed a generalized scarlatiniform eruption most pronounced in the body and limb folds. The only abnormality noted in the mouth was a mild coated tongue. There was no conjunctival injection. She was not menstruating and had no vaginal discharge. There was no evidence on the skin or from general examination of an infective focus. Blood pressure was 120/80. A full blood count showed a total white cell count of $12{\cdot}6 \times 10^9/l$ with a neutrophil leukocytosis. The ESR was normal. She was commenced on intramuscular penicillin. After 36 h her temperature had fallen and her general condition improved. In another 2 days the rash commenced to peel and desquamation last involved the feet 2 weeks later. Throat, vaginal and blood cultures were subsequently sterile. Paired streptococcal antibodies including ASO, AHT, ASK and anti-DNAase B were normal.

No diagnosis of the illness was made. There was no convincing evidence for scarlet fever, toxic shock syndrome or a drug reaction and idiopathic recurrent pseudo-scarlatina[1] was

Correspondence: Dr M.B.Duffill.

suspected. However, in case an unrecognized drug was the cause she was instructed to take no further over-the-counter medications of any kind.

During the remainder of 1985 she had six further similar attacks for which again no cause could be found. A neutrophil leukocytosis and normal ESR were recorded on three further occasions. Repeated throat swabs and paired streptococcal antibodies were normal. Cultures from her skin surface, including the classical staphylococcal carrier sites and vagina grew normal flora only. Gynaecological and ear, nose and throat examinations revealed no evidence of occult infection.

Although all self-medications had been prohibited, codeine allergy was next suspected because of reports of this drug causing seemingly identical pseudo-scarlatina attacks to those in our patient.[2,3] However, a patch test and oral challenge to codeine were negative. From this time her attacks ceased.

In late 1986 she experienced a further attack after taking a little Nyal Decongestant Cough Elixir (Adult) New Formula which she had just adminstered to her daughter. She then challenged herself with this medicine and reproduced an attack within hours. She was then challenged orally with components of the Elixir as supplied by the manufacturer at the same concentration as in the original preparation. She developed a typical attack within 5 h of challenge with 5 ml of 0·5 g/100 ml aqueous solution of pseudoephedrine hydrochloride. Challenge with the other components of the mixture which included codeine phosphate, was negative. A patch test with a 1% aqueous solution of pseudoephedrine hydrochloride was negative.

On repeat questioning about past attacks, the patient then recalled that her first attack 13 years previously had been due to Actifed, which contains pseudoephedrine hydrochloride and triprolidine hydrochloride. However, she denied taking any pseudoephedrine-containing preparations during the series of attacks in 1985, and there was no reliable recollection of medications associated with the attacks prior to this, other than the first. There have been no further attacks at the time of writing and she now avoids all pseudoephedrine-containing preparations.

DISCUSSION

Skin reactions to pseudoephedrine are apparently rare and we have been able to find only one such previous report.[4] The report does not specify the morphology of the rash, but states that it was severe, generalized, and accompanied by rigors, joint swelling, faintness and tachycardia.

Recurrent idiopathic pseudo-scarlatina is a well-documented entity.[1] However, codeine has been described as causing a very similar clinical picture.[2,3] Codeine is not generally recognized as causing exanthems, particularly in association with fever. In view of this, it is not surprising that diagnosis of most reported cases of pseudoscarlatina due to codeine has been considerably delayed. It is of interest, therefore, that pseudoephedrine, which also is often present in symptomatic remedies for colds and coughs, was observed to cause pseudo-scarlatina in our patient. Itch was not a prominent feature in our patient's rash as it has been in most reported codeine exanthems. Also a patch-test with pseudoephedrine hydrochloride was negative whereas most reported cases of pseudo-scarlatina due to codeine have exhibited positive patch-tests to codeine (and also to morphine).[3] The series of attacks the patient had in 1985 must be regarded as idiopathic, although the possibility exists that she was taking pseudoephedrine in some unrecognized form.

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Society Proceedings

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SUMMARIES OF PAPERS

Airborne irritant contact dermatitis from industrial dust particles

J.M.LACHAPELLE

Occupational Dermatology Unit, Catholic University, Louvain-Brussels, Belgium

Airborne irritant contact dermatitis caused by industrial dust particles is common. The symptoms are quite characteristic: pruritus, excoriations, papules and pustules. There even may be prurigo-like lesions. Often these symptoms are more pronounced in atopic individuals. Several investigations can help in establishing the diagnosis. These are: detection of particles on the skin and examination of their morphology in a skin surface biopsy under polarized light, determination of pH of a suspension of airborne particles and determination of the relative humidity in the ambient air. Experimental studies showed the limited efficacy of silicone barrier creams.

Diagnostic immunofluorescence in dermatology

(in memoriam Prof. Dr R.H.Cormane: 7/4/1925–18/2/1987)

M.C.J.M.DE JONG

Department of Dermatology, University Hospital, Groningen

In 1964 Rudy Cormane published his pioneering work on diagnostic immunofluorescence (IF) microscopy as applied to the field of dermatology.[1] In the late sixties, he was again in the lead using a new type of vertical illuminator permitting incident light fluorescence. This instrument not only proved to be more sensitive than the transmitted light system, but also created the possibility of advanced double wavelength fluorescence.

It was not until 1982, however, that a procedure was described employing red (TRITC) and green (FITC) labelled conjugates to localize simultaneously two different antigens in skin sections.[2] This procedure aimed at a rapid and relatively inexpensive immunohistopathological diagnosis of certain bullous skin diseases. Essentially it attempts to assess at which level IgG or IgA auto-antibody, and bulla formation, are localized in the dermo-epidermal junction.

Double IF staining procedures using antisera against laminin and collagen type IV are now the most important tool in differentiating problem cases such as epidermolysis bullosa variants and linear IgA bullous dermatoses. According to the technique described by Gammon *et al.*,[2] green and red labelled structures are both observed at the same wavelength, resulting in yellow-orange instead of red fluorescence of the TRITC tracer. However, yellow-orange emission may lead to erroneous interpretation since overlap of red and green labelled antigens may also produce various shades of yellow-orange fluorescence. This applies in particular to the dermo-epidermal junction expressing closely assembled antigens such as laminin, collagen type IV, BP antigen and EBA antigen. Therefore, separate observation of red and green antigen tracers at their respective excitation and emission optima is preferable.

The overall result to be interpreted with this selective 'double wavelength' procedure requires photomicrographic recording. Notwithstanding problems of colour shifting with the double recording technique, it appears feasible to distinguish shades of red, green and yellow-orange. The colour yellow-orange in such recordings should umambiguously indicate topographical overlap of both red and green marked antigens. It is even feasible to introduce a third fluorochrome, e.g. a blue emitting DNA tracer which may help to identify the presence of inflammatory cells. The overall result, visualized in 'triple recordings', may yield not only attractively coloured but also diagnostically informative pictures.

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Pathogenesis of chronic urticaria and angio-oedema

H.M.G.Doeglas

Department of Dermatology, University Hospital, Groningen

The mast cell is still considered to play a central role in the genesis of urticaria. It can be triggered to release mediators by allergic mechanisms of type I and type III, and by pseudo-allergy as in the case of chemical histamine release and physical urticaria.

To be able to ascribe urticaria to type I allergy it is necessary to have a combination of history (pointing to a specific antigen), intracutaneous skin tests or RAST (with this antigen) and, preferably, a positive (placebo controlled) provocation test. Type III allergy is harder to demonstrate, although the following are helpful: histopathological and immunofluorescence findings, evidence of circulating immune complexes and evidence of certain infections, systemic diseases or drug intolerances known to be associated with type III allergy. Examples of infections are: hepatitis, infectious mononucleosis, tropical worm- or parasitic infections and perhaps certain 'focal infections'. Relevant systemic diseases are SLE and other auto-immune diseases (thyroid), and haematological diseases such as leukemia polycythaemia (M.Kahler, M.Hodgkin and M.Waldenström).

Pseudo-allergy of a chemical nature involves histamine release by chemicals such as X-ray contrast materials, plasma expanders, opiates and curare like drugs. These reactions are usually the cause of acute urticaria.

Physical urticaria can be considered a form of pseudo-allergy also. Although there are many types of physical urticaria, the following four types occur most frequently: urticaria factitia, delayed pressure urticaria, cold contact urticaria and cholinergic (heat reflex) urticaria. Together they comprise about 90% of all patients with physical urticaria. The differentiation of the various types of physical urticaria is of the utmost importance to the patient. It enables the physician to give a prognosis and to advise the patient how to avoid causative factors and under what circumstances to use antihistamines or other drugs. In our experience physical urticaria is present in about 50% of patients who present with chronic urticaria (Table 1). Intolerance of analgesics is considered a secondary, exacerbating cause of urticaria and angio-oedema, occurring in all types of urticaria including physical urticaria. It is not limited to aspirin, but includes propyphenazone derivatives and anti-rheumatic drugs also; paracetamol is frequently well tolerated. Oral provocation tests should only be undertaken under strict surveillance in hospital. The effect is dose related.

TABLE 1. Chronic urticaria. Primary diagnosis in 633 patients, seen during the period 1975–1981

Type of urticaria	No. of patients	%
Chronic idiopathic urticaria*	247	39.0
Chronic idiopathic angio-oedema*	48	7·6
Urticaria factitia	95	15·0
Delayed and persistent dermographism	14	2·2
Delayed pressure urticaria	57	9·0
Cold contact urticaria	67	10·6
Other types of cold urticaria	6	0·9
Heat contact urticaria	4	0·6
Cholinergic urticaria	63	10·0
Light urticaria	2	0·3
Other types of physical urticaria	6	0·9
Acquired C_1 esterase inhibitor deficiency	1	0·2
Type 1 allergic urticaria	15	2·4
Other types of urticaria	8	1·2
Total	633	100·0

* Including type III allergy

Pseudomalignant lymphomatoid skin reactions due to drugs

S.H.KARDAUN

Department of Dermatology, University Hospital, Groningen

Pseudolymphoma syndrome was first reported in the literature in 1940.[1] Generally, this is considered to be a hypersensitivity reaction, nearly always due to anticonvulsant drugs. This syndrome is characterized by the triad of fever, a generalized rash and lymphadenopathy and may be accompanied by other symptoms, e.g. eosinophilia and arthralgia. In this syndrome the histological features of the enlarged lymph nodes may be difficult to distinguish from those seen in malignant lymphomas. Histological investigations of the skin are seldom mentioned in the literature and can be non-specific.[2] Some case reports describe a histological picture of mycosis fungoides.[3,4]

In a prospective study with well defined drug-induced skin rashes,[5] we found in the skin of five out of 80 cases a histological picture suggestive of malignant lymphoma. In four of these we found a mycosis fungoides-like picture with epidermotropism and cerebriform mononuclear cells, while in two patients Pautrier microabscess-like structures were also seen. The patients presented with different clinical pictures: two had generalized and three localized skin rashes. They had used various drugs. The causative agents in four of them did not belong to the anticonvulsants. Cessation of the suspected drugs in all cases led to a rapid healing of the lesions.

Thus the histological picture of the skin in drug rashes can mimick a malignant lymphoma, and drugs not belonging to the anticonvulsants may induce pseudomalignant lymphomatoid skin reactions.

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Localization of spirochaetes in lymphadenosis benigna cutis and erythema chronicum migrans

J.DE KONING AND A.J.VELDERS

Laboratory for Public Health, Leeuwarden

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Side-effects of cosmetics: the role of contact allergy

A.C.DE GROOT, E.BEVERDAM, C.TJONG AYONG, P.J.COENRAADS AND J.P.NATER

Willem Alexander Hospital, 's Hertogenbosch and Department of Dermatology, University Hospital, Groningen

To be published elsewhere.

Chlamydia trachomatis infection: (Sero) diagnosis and pelvic inflammatory disease

P.C.VAN VOORST VADER

Department of Dermatology, University Hospital, Groningen

To be published elsewhere.

CASE REPORTS

Localized bullous pemphigoid

S.H.KARDAUN, M.C.J.M.DE JONG AND R.A.TUPKER

Department of Dermatology, University Hospital, Groningen

History. Male, aged 70 years. His left lower leg was amputated in November 1984 because of severe cellulitis following incorrect healing of a fracture. About 2 weeks after exercising with a prosthesis, he developed blisters on the amputation stump and later on also on his right foot. The bullae disappeared after discontinuing the training, but reappeared after restarting exercises.

Examination. On the stump, the lateral side of the right foot and dorsa of the toes, tense bullae (diameter 1–3 cm) on an erythematous base were noted (Fig. 1). The genital and oral mucosae showed no abnormalities.

Laboratory tests. ESR 35 mm/h, peripheral leukocyte differential count: eosinophils 13%, LDH 317 U/l. Rose/latex, ANA, ANF, anti-dsDNA and APCA were negative. Patch testing with the European standard series (ICDRG) and prosthesis material was negative.

Histology. Edge of a bulla: a rather acanthotic, otherwise normal epidermis covered a subepidermal bulla, filled with erythrocytes and eosinophils. The upper dermis contained a dense inflammatory infiltrate with many eosinophils.

Immunofluorescence. Edge of a bulla: junctional splitting and vesicle formation at the epidermal basement zone with continuous linear IgG and C_{3c} deposition. Double IF staining with anti-IgG, anti-laminin and anti-collagen type IV localized the respective antigens mainly to the floor of the blisters. Circulating IgG anti-BMZ antibodies were detected at a titre of 1:5120 (on monkey oesophagus).

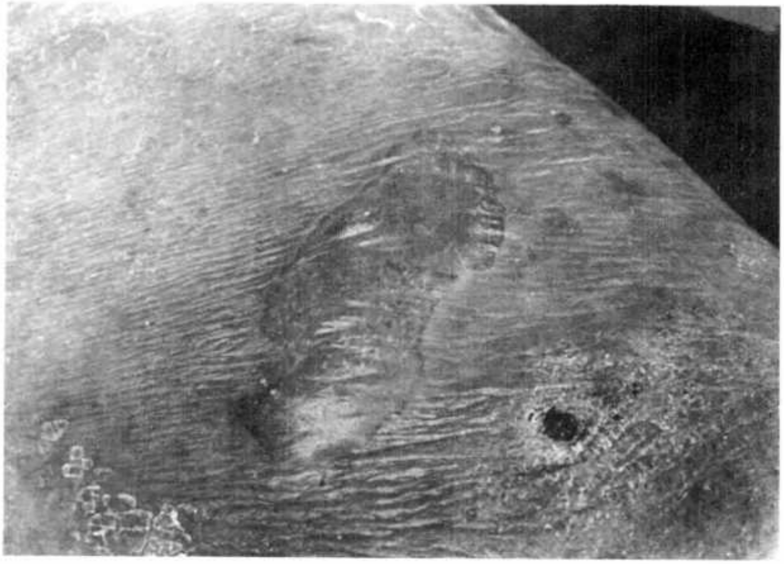

FIGURE 1. Tense bulla on an erythematous base on the amputation stump.

Treatment. Prednisone (40 mg orally daily) combined with the use of a Thomas splint (minimalizing pressure on the stump) instead of a prosthesis resulted in disappearance of the blisters within 3 weeks. After tapering off and finally stopping prednisone after 9 months, the patient remained symptom free.

Discussion. To differentiate between bullous pemphigoid (BP) and epidermolysis bullosa acquisita (EBA) a double IF staining technique was employed. In BP both IgG and laminin are found on the floor of the blister, whereas in EBA these antigens are localized in the roof of the blister.[1] Our findings are consitent with the diagnosis of localized BP.[2,3]

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Orogenital lichenoid (drug) reaction

P.C.van Voorst Vader, S.H.Kardaun, R.A.Tupker, M.C.J.M.de Jong,
L.G.M.de Bont* and F.M.Kauer†

Departments of Dermatology, * *Oral Surgery and* † *Gynaecology, University Hospital, Groningen*

History. Female, aged 51 years. Since 1978 she had suffered from erosive, painful, vulvar lesions. Since 1984 there were concomitant painful, gingival lesions. In 1985 lesions appeared on the wrists and in 1987 on the feet. Medication: 1977–1983 hydrochlorothiazide, 1983–March 1987 metoprolol.

Examination. Mucous membrane of vulva and gingiva: diffuse erythema with erosions. Buccal mucous membrane: flat, white, linear lesions in a reticular pattern. Flexor aspects of wrists and medial and lateral side of the feet: salmon-red, flat, polygonal papules with barely discernible striae of Wickham.

Histology. Vulvar, gingival and buccal mucous membrane: the epithelium showed foci with a well-developed stratum granulosum and colloid bodies and a stratum basale with liquefaction necrosis and cleft formation; in the lamina propria adjacent to the epithelium, a band-like lymphohistiocytic infiltrate with a few eosinophilic granulocytes and slight exocytosis was present. Papule on the wrist: the epidermis showed slight thickening of the stratum granulosum and a stratum basale with foci with colloid bodies and liquefaction necrosis; in the papillary dermis a patchy lymphohistiocytic infiltrate with slight exocytosis and some melanophores was present.

Immunofluorescence. Gingiva: clusters of IgM positive colloid bodies in the epithelial-subepithelial junction area. Papule on the wrist: the epidermis showed vacuolization with extensive clusters of IgM and C_{3c} positive necrotic keratinocytes and colloid bodies; in the dermo-epidermal junction zone a more or less homogeneous deposition of fibrin was observed (Fig. 2).

Course. Definite amelioration was seen after discontinuation of the metoprolol, although a complete remission was not seen during a follow-up period of three months. A provocation test was not performed.

Discussion. An erosive variant of lichen planus localized to the vulva and the gingiva has been described.[1,2] The immunofluorescence findings (numerous intraepidermal necrotic keratinocytes and colloid bodies and homogeneous rather than frayed junctional fibrin deposition)

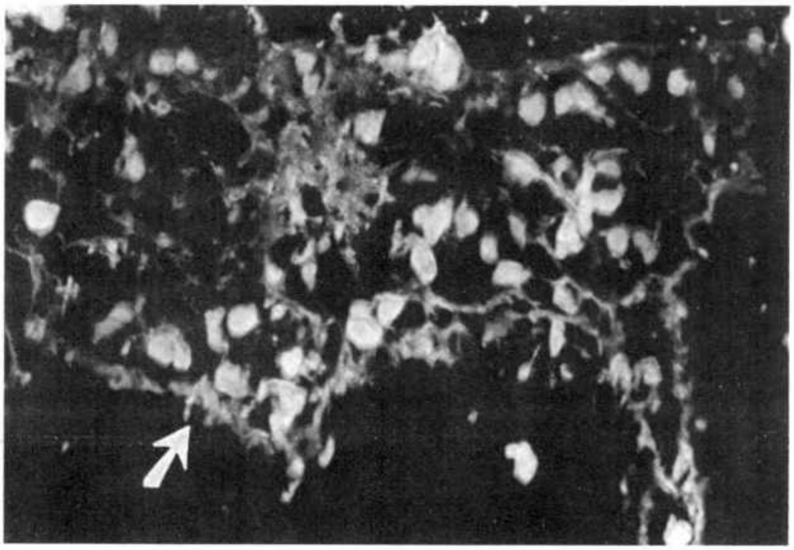

FIGURE 2. Cryostat section of lesional skin. Double immunofluorescence staining shows intraepidermal IgM positive colloid bodies and homogeneous fibrin deposition at the dermo-epidermal junction (arrow).

suggest a lichenoid drug reaction. Beta-blockers are known to be a cause of lichenoid drug reactions.[3] The clinical course does not exclude the possibility of a lichenoid drug reaction superimposed on lichen planus.

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Infantile haemangiomatosis with involvement of skin, liver and placenta

P.MEIJER, M.F.HASPER, C.M.A.BIJLEVELD*, P.C.VAN VOORST VADER AND P.WARNAAR†

*Departments of Dermatology and *Paediatrics, University Hospital, Groningen and † Department of Dermatology, Hospital Meppel*

History. Boy, aged 4 years. At birth many red raised spots were present on the skin, and after birth new lesions appeared daily. Treatment for prolonged jaundice (neonatal icterus) with phototherapy was unsuccessful.

Examination. Multiple red macules and papules with a smooth or irregular surface, 1–4 mm in diameter, were seen all over the skin (Figs. 3 and 4). Except for jaundice his overall condition was good. There were no signs of congestive heart failure.

Laboratory tests. Liver function tests pointed to an obstructive icterus (high fraction of conjugated bilirubin). ESR, full blood count, electrolytes and renal function tests were normal, as were a thrombocyte count and coagulation tests. Tests for infection and metabolic disorders were negative.

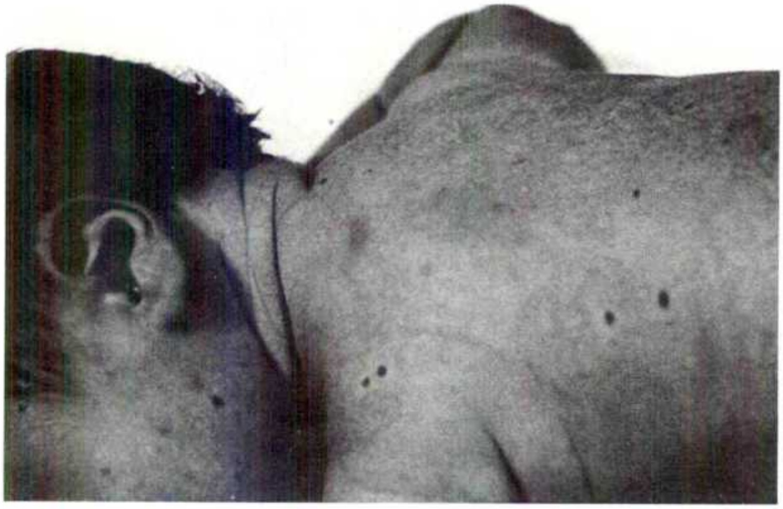

FIGURE 3. Multiple red macules and papules, 1–4 mm, with a smooth or irregular surface.

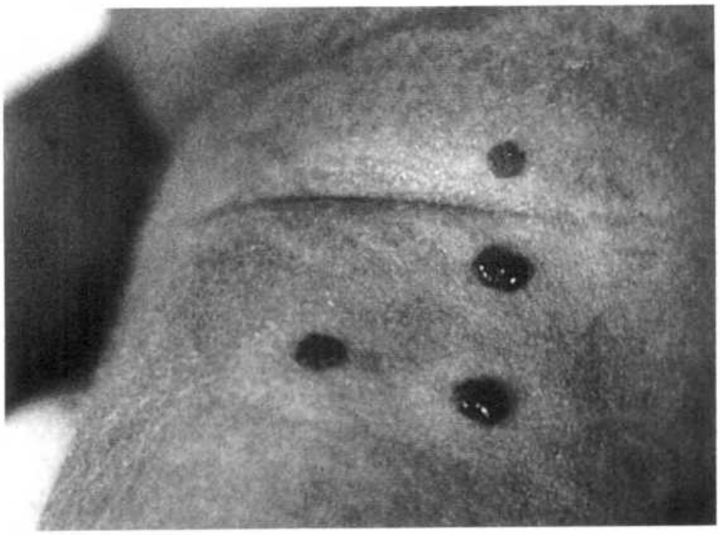

FIGURE 4. Close-up of haemangiomas.

Investigations. To exclude extrahepatic bile duct atresia, an exploratory laparotomy was performed at the age of 2 months, which showed normal bile ducts, but multiple haemangiomas in the liver (not seen by laparoscopy).

Histology. The skin lesions showed, under a normal epidermis, groups of wide capillaries with somewhat swollen endothelial cells in the papillary dermis. A liver biopsy showed cholestase and inflammatory infiltrates in the portal fields. On the fetal side of the placenta the histological picture was that of a capillary haemangioma.

Treatment. Some cutaneous lesions were treated with an Argon laser, leaving small scars.[1] Because there was no improvement in the liver function tests after 10 weeks, systemic corticosteroids were given for several months to reduce the risk of cirrhosis. This resulted in total regression of the skin lesions within 5 months, without scarring. The liver function tests became normal after 1 year and remained normal thereafter. Psychomotor development was normal.

Discussion. Infantile (juvenile) haemangiomas are mostly confined to the skin or the subcutaneous tissues, but occasionally involve other organs. Spontaneous regression of the haemangiomas is well known, but serious complications caused by bleeding and obstructions are possible. In this patient the cholestasis was probably caused by the haemangiomas.[2,3] Because of undetected haemangiomas, invasive procedures like liver biopsies can be dangerous.

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Tuberous sclerosis

M.F.HASPER AND P.MEIJER

Department of Dermatology, University Hospital, Groningen

History. Female aged 27 years. Since the age of 3 years there were progressively spreading red papular lesions on the face. General health was good, except for epilepsy since childhood. The family history was negative for neurological disorders or skin diseases.

Examination. Numerous firm red-brown papules, 3–8 mm in diameter were present symmetrically on the cheeks, nose, chin and forehead. The neck and axillae showed numerous soft brown pedunculated fibromas. On the trunk and extremities solitary red-brown papules were seen as well as several discrete oval hypopigmented macules, 1–10 cm diameter. Numerous pretibial freckles were seen. A periungual filamentous excrescence (Koenen's tumour) was visible on one of the toes. The oral mucosa showed multiple firm papillomas (Fig. 5). In all teeth, pin-point pits (enamel defects) were present, several pits per tooth (Fig. 6).

Histology. Papule on scapula: compatible with angiofibroma.

Laboratory tests. Blood count, ESR, serum glucose, thyroxine, renal and liver function tests were all normal. X-ray of the skull showed patchy intracranial calcifications. X-rays of the chest and hands were normal, as was echography of the kidneys.

Treatment. The lesions on the face and neck were treated with electrocoagulation and excochleation with satisfactory results.

Discussion. In 1975 Hoff *et al.*[1] described a new symptom in this disease: small pit-shaped pin-point defects in the dental enamel. These findings have been confirmed by Valkilzadeh and

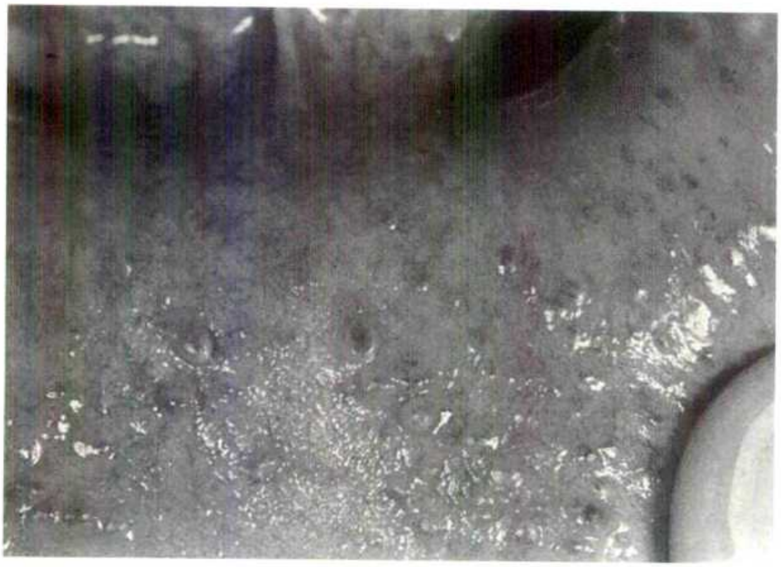

FIGURE 5. Multiple firm papillomas on the oral mucous membrane.

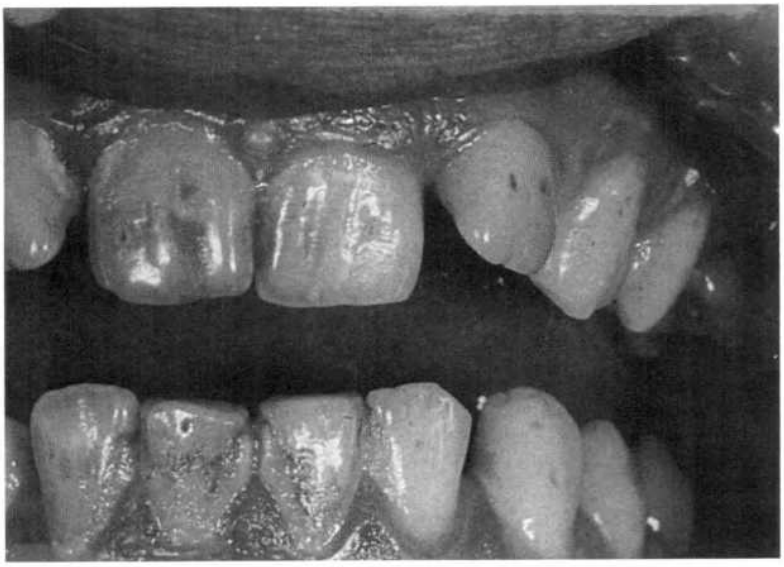

FIGURE 6. Pathognomonic pin-point pits in the dental enamel.

Happle.[2] The pits are found in both deciduous and permanent dentition, varying from 1–11 pits per tooth, with diameters up to 100 μm. These enamel hypoplasias are important in the early diagnosis, as they are pathognomonic and can be found in all patients from the age of 6 months, when the first tooth breaks through.

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Cicatricial fibromatosis

P.C.VAN VOORST VADER, C.C.M.VAN GERVEN, S.H.KARDAUN, J.W.OOSTERHUIS* AND E.W.SAUER†

*Departments of Dermatologyy and * Pathology, University Hospital, and † Department of Plastic Surgery, Catholic Hospital, Groningen*

History. Female, aged 41 years. For 1 year a slowly enlarging skin tumour had been present at the site of a scar (probably caused by a drain) due to a ductus Botalli operation at the age of 6 years.

Examination. On the left shoulder there was a lesion, 3 × 3 cm, showing partly a depressed, atrophic and red-brown area and partly an indurated, slightly elevated, mostly skin-coloured

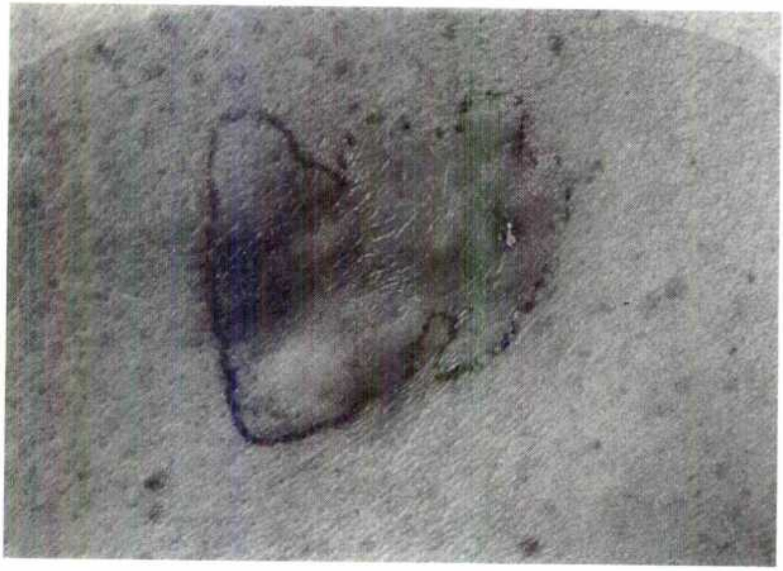

FIGURE 7. Lesion on the left shoulder, 3 × 3 cm in size.

and partially light-brown tumour with a highly irregular border (Fig. 7). Other scars did not show any abnormalities.

Histology. Excision biopsy: a richly cellularized dermal lesion, deeply infiltrating the subcutis, hard to delineate, mostly consisting of elongated, spindle-shaped, fibroblastic cells, arranged in bundles, without atypia or mitoses. The nuclei contained one or more minute nucleoli. Between the cells an abundant amount of collagen was observed.

Treatment. Wide excision, including the subcutis down to the fascia. No recurrence was seen during a follow-up period of 2 years.

Discussion. Cicatricial fibromatosis forms a subgroup of an entity known as extra-abdominal fibrosis, which is a rare, non-metastasizing, proliferative disorder arising from connective tissue of muscle and overlying fascia or aponeurosis (musculoaponeurotic fibromatosis), chiefly affecting the muscles of the shoulder and pelvic girdle and the thigh of adolescents and young adults and occurring with equal frequency in both sexes.

Aggressive, deep, local infiltration of extra-abdominal fibromatosis can occur and may have serious consequences such as the vena cava superior syndrome. In 13 out of a series of 367 cases the lesion arose from a surgical scar (cicatricial fibromatosis).[1] The occurrence in a 35-year old scar, the irregular clinical border and the histological growth pattern with infiltration deep into the subcutis of a richly cellularized lesion suggest that the lesion in this case is unlikely to be a keloid, but should be diagnosed as cicatricial fibromatosis.

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Epithelioid sarcoma

S.H.KARDAUN, M.H.KRUIS-DE VRIES, J.W.OOSTERHUIS*, H.M.G.DOEGLAS AND M.F.HASPER

*Departments of Dermatology and *Pathology, University Hospital, Groningen*

History. Male, aged 23 years. For 6 months he had suffered from an ulcer on his right lower leg, not responding to local therapeutic measures.

Examination. Pretibial on the right lower leg, an ulcer, 3 × 3½ cm, with central deep necrosis and smooth, slightly elevated, undermined margins was present (Fig. 8). There were no palpable lymph nodes.

Histology. Two consecutive punch biopsies showed a dense polymorphous dermal infiltrate with granulation tissue. They were not diagnostic. Excision: beneath an acanthotic epidermis, the dermis was extensively infiltrated. Besides a dense polymorphous infiltrate with granulation tissue the dermis contained fields and strings of epithelioid cells and some central necrosis. The tumour cells showed eosinophilic cytoplasm, atypical nuclei and atypical mitoses (Fig. 9). Within the tumour hyaline material was also found.

Immunohistochemically the tumour cells stained positively for vimentin and cytokeratin. Electron microscopy revealed paranuclear clusters of these intracytoplasmic intermediate filaments.

Treatment. Complete excision was performed because of the persistence of the ulcer, for which we had no explanation. Histological examination finally resulted in the diagnosis. Five

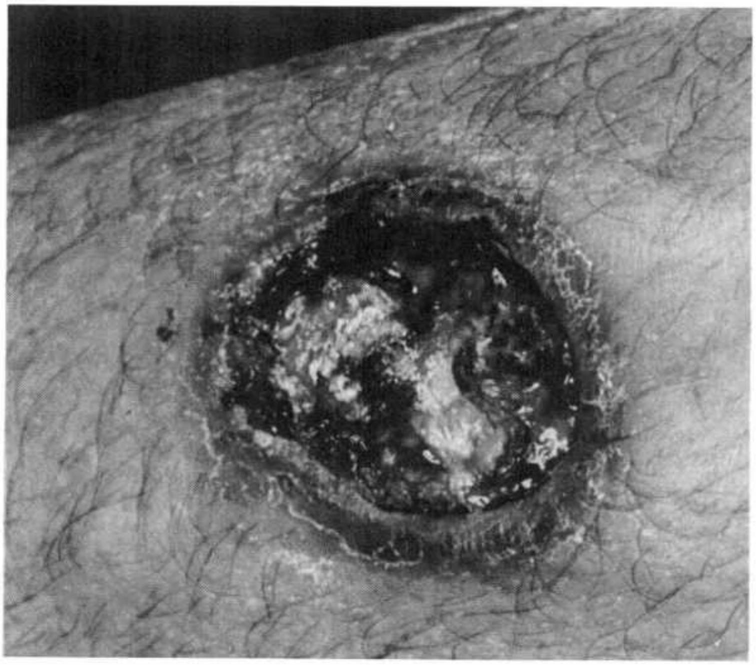

FIGURE 8. Pretibial ulcer with central deep necrosis and smooth, elevated, undermined margin.

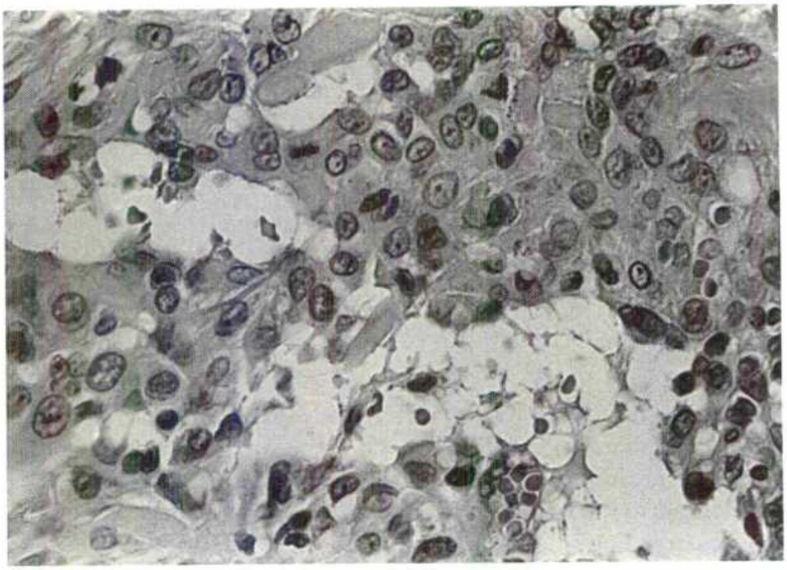

FIGURE 9. Epithelioid cells showing eosinophilic cytoplasm, atypical nuclei and atypical mitoses.

months later a large inguinal lymph node infiltrated with metastatic tumour cells was found. The lymph node was excised followed by local perfusion with melfalan and actinomycin.

Discussion. Epithelioid sarcoma was first described by Enzinger in 1970.[1] It is a rare tumour, mostly occurring in young adults. Men are more frequently affected than women. The tumour is preferentially located on the distal parts of the lower and upper limbs. Histologically the tumour is often difficult to recognize,[2,3] resembling several benign and also some malignant processes. The excised material finally enabled us to establish its real nature. Thus, in atypical ulcers with unknown etiology wedge excisions are recommended in order to provide the correct diagnosis. Surgical intervention is the treatment of choice. Recurrences of the tumour are seen in 77% of cases and metastases in 45%, mainly in the lymph nodes and lungs. A prolonged follow-up is indicated, because recurrences of the tumour have been reported after as long as 19 years.

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Lymphomatoid granulomatosis of the skin

S.H.KARDAUN, C.C.M.VAN GERVEN, J.W.OOSTERHUIS*, M.C.J.M.DE JONG AND W.H.BOS†

*Departments of Dermatology and *Pathology, University Hospital, Groningen and †Department of Dermatology, Hospital Leeuwarden*

History. Male, aged 83 years. In 1985 he was hospitalized because of oedematous legs and

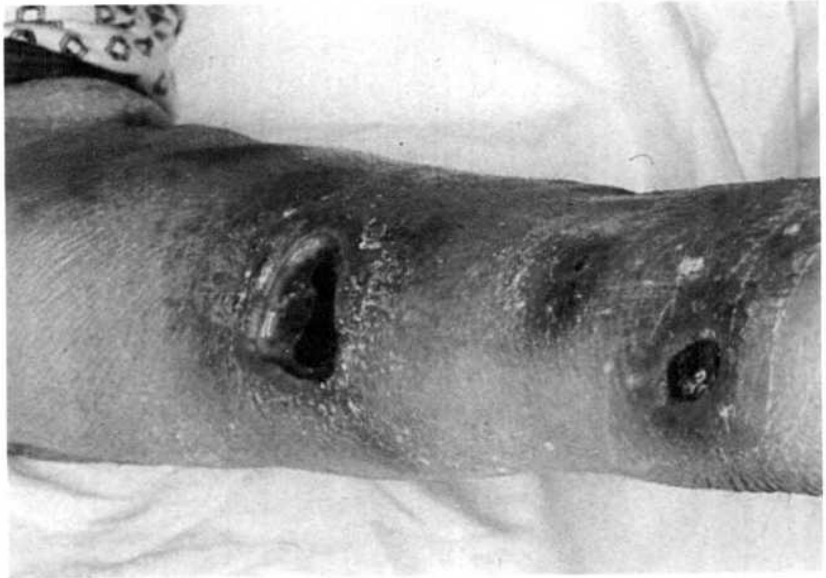

FIGURE 10. Firm, purplish-red nodules on the forearm, some showing deep, central necrosis.

hepatomegaly e.c.i. with painless nodular skin lesions on the feet, interpreted as leukocytoclastic vasculitis. In 1986 he was rehospitalized because of progression of the skin lesions with ulceration.

Examination. On his arms, legs and feet he had 1–7 cm purple-red, firm, painless nodules. Some of these showed central ulceration, sometimes down to the subcutis or even into the muscle (Fig. 10).

Histology. 1985: Epidermis: normal. Dermis and subcutis: perivascular and periadnexal, dense, polymorphous infiltrates, consisting of lymphocytes, histiocytes, some neutrophils and eosinophils. The deeper dermis and subcutis demonstrated a leukocytoclastic vasculitis, with nuclear dust, neutrophils, fibrinoid necrosis and extravasation of erythrocytes. The subcutis also showed necrosis and naked giant cells. 1986: The picture was largely the same as in 1985, but infiltrates partly invaded the sweat glands, demonstrated a more monomorphous character and contained atypical lymphoid cells of T helper phenotype (CD4+, CD8−) with cerebriform nuclei, distinct nucleoli and several atypical mitoses (Fig. 11). Conclusion: lymphomatoid granulomatosis with progression to pleiomorphic T cell lymphoma.

Immunofluorescence. Lesion, 1986: the dermo-epidermal junction zone showed linear IgM(3+);[1] the vessel walls in the mid and deeper dermis showed extensive deposition of fibrin (3+/4+), whereas some vessel walls also showed IgM(2+) and C_{3c}(3+) deposits. Normal skin: no abnormalities. The findings are consistent with immune complex vasculitis.

Treatment. Cyclophosphamide 2 mg/kg/day and prednisolone 1 mg/kg/day resulted in slow healing of the ulcers. During therapy the patient suffered from peritonitis due to perforating diverticulitis, and therefore, we had to interrupt treatment. After several weeks the patient died, probably due to pulmonary embolism. Autopsy was not performed.

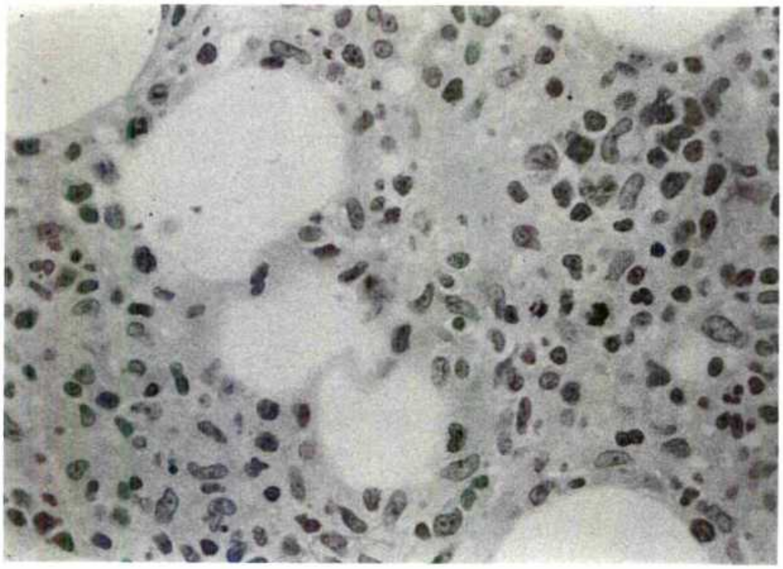

FIGURE 11. Monomorphous lymphoreticular infiltrate in the subcutis showing atypical lymphoid cells with clearly visible nucleoli and atypical mitoses.

Discussion. Lymphomatoid granulomatosis was first described by Liebow in 1972.[2] The skin lesions are sometimes painful and ulcerating and mostly consist of erythematous papules, subcutaneous or dermal nodules or plaques.[3] It is a rare disease, occurring more in men than in women. It manifests itself mainly in the lungs, the central nervous system and the skin. Histologically the disease is characterized by a vasculitic, angiocentric and angiodestructive lymphoreticular process, combined with a polymorphous, partly granulomatous infiltrate showing cellular atypia. In our patient, despite intensive investigations, only skin lesions were found. Skin lesions as the sole manifestation of the disease are reported in 10% of cases. The prognosis is variable; untreated, mortality is high. Early and prolonged treatment with cyclophosphamide and prednisolone may result in long and even complete remissions. However, in 12–46% malignant T cell lymphoma will develop.

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Cutaneous immunoblastic T cell lymphoma

P.C.VAN VOORST VADER, S.POPPEMA* AND S.DAENEN†

*Departments of Dermatology, * Pathology and † Haematology, University Hospital, Groningen*

History. Male, aged 49 years. He had been suffering from cutaneous T cell lymphoma since the age of 35. Cutaneous tumours were first observed at the age of 40, when intentionally

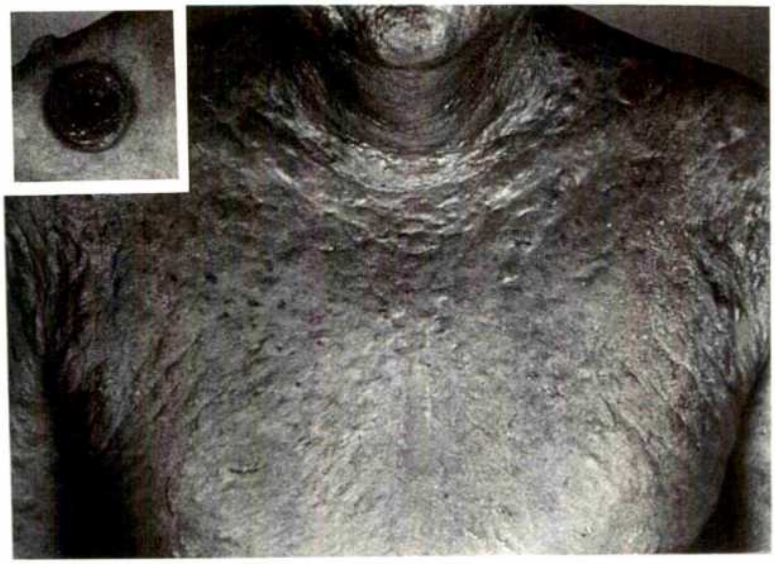

FIGURE 12. Massive cutaneous infiltration of the chest at the age of 44. Inset: recurrent tumour during maintenance therapy with systemic steroid and retinoid.

curative treatment by total skin electron beam irradiation (3500 rad) resulted in a complete remission for 1 year. At the age of 44 a massive recurrence (Fig. 12) was successfully treated by palliative total skin electron beam irradiation (1500 rad) as phototherapy plus oral retinoid therapy was no longer effective. Maintenance therapy for recurrent tumours was then started with weekly doses of 500–1000 mg methylprednisolone i.v. and oral etretinate, 50 mg daily. Tumours not responding to this maintenance regimen were given radiotherapy. Extracutaneous involvement was never demonstrated, apart from a subcutaneous perianal tumour at the age of 48, which also responded to radiotherapy.

Examination. The skin showed some erythematous plaques and several, partly erosive, tumours (Fig. 12, inset), especially in the inguinal area. No palpable lymph nodes were present.

Histology. Over the years all biopsies showed essentially the same picture: epidermal exocytosis, sometimes resulting in Pautrier micro-abscesses, and a dense dermal infiltrate composed of varying numbers of immunoblastic cells with distinct central nucleoli and frequent mitoses, lymphoid cells, sometimes showing cerebriform nuclei, and histiocytic cells (Fig. 13). At the age of 44 the immunoblastic cells of a cutaneous tumour were T1 (pan T cell), T3 (pan T cell), T4 (helper T cell), T6 (thymocyte) and T11 (E rosette receptor) positive. T8 (suppressor T cell) positive immunoblastic cells were also observed. At the age of 48 the immunoblastic cells of the subcutaneous tumour were only T1 and T4 positive.

Treatment. Maintenance therapy with weekly methylprednisolone i.v. and daily oral etretinate with additional radiotherapy for non-responding cutaneous tumours was continued.

Discussion. This patient had had a cutaneous immunoblastic T cell lymphoma for 14 years, having reached the cutaneous tumour stage 9 years ago, without ever developing extracutaneous involvement.[1] Maintenance therapy with a systemic steroid and retinoid appeared to be moderately effective. It is not clear whether the T4 and T8 positive immunoblastic cells

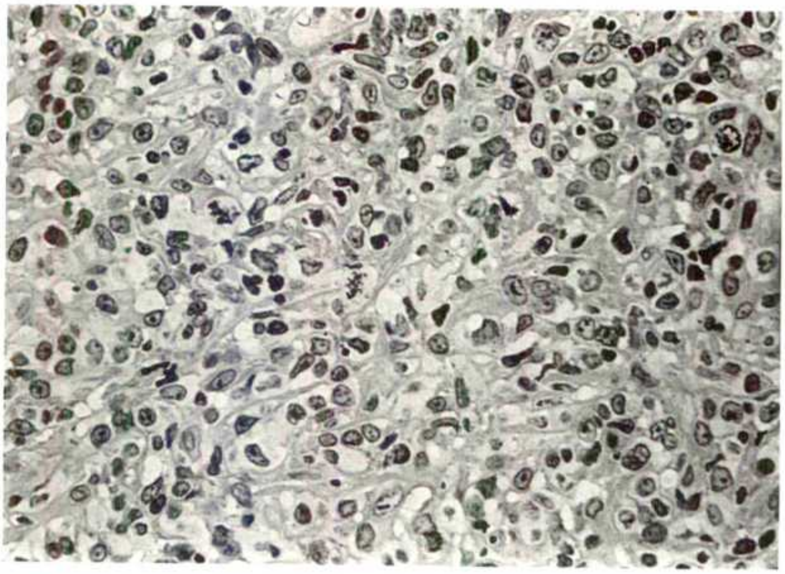

FIGURE 13. Dense dermal infiltrate with a large number of immunoblastic cells with central nucleoli.

consisted of a polyclonal or a monoclonal population, as double staining or gene rearrangement studies were not performed.[2]

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Linear fixed drug eruption

MADAM, The exact reason for the localization of fixed drug eruptions to certain skin sites remains unknown. We would like to report a case of fixed drug eruption, appearing in a striking linear pattern on the lower limb, following a neural distribution or Blaschko's lines, secondary to intramuscular injections of cefazoline (cephazolin).

A 59-year-old-man, born in Algeria, was first seen in our department in October 1986 with an eruption that had begun 3 days earlier. He had received intramuscular injections of cephazolin in the buttocks (2 g per day for 8 days) and nasal sodium cromoglycate, for an influenza-like illness. He reported a scrotal eruption 2 months earlier after administration of an unknown drug. Clinical examination revealed numerous erythematous and hyperpigmented macular lesions, 4–5 cm diameter, with flaccid blisters or round erosions on some of them. The lesions were mainly located on the left leg in a striking linear pattern, extending from the left buttock down the posterior aspect of the thigh and the leg and to the outer aspect of the dorsum of the foot (Fig. 1); the sole was unaffected. A few other lesions were found on the other leg, the backs of the hands, the upper lip and the scrotum. The patient complained itching and burning. No other

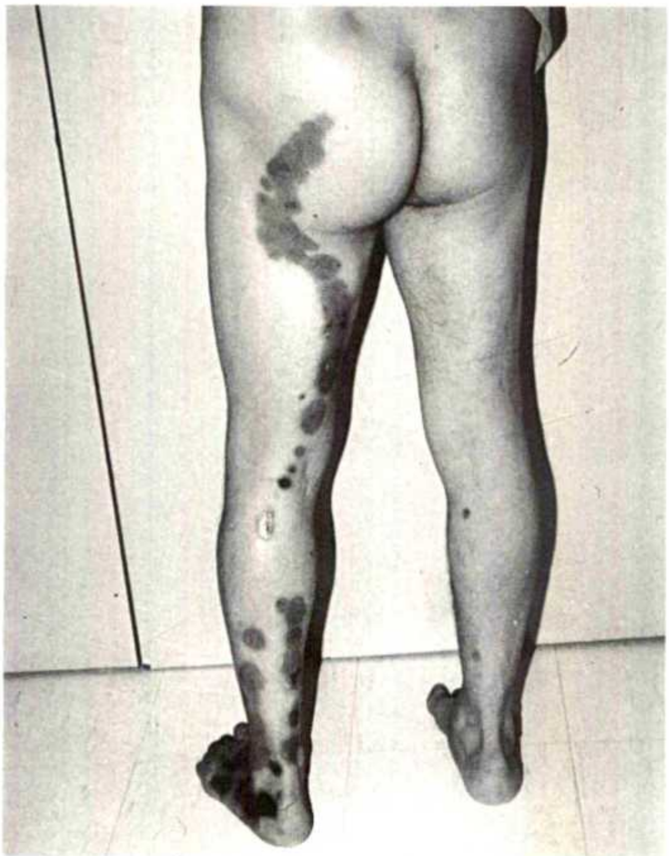

FIGURE 1. Linear eruption on the left lower limb.

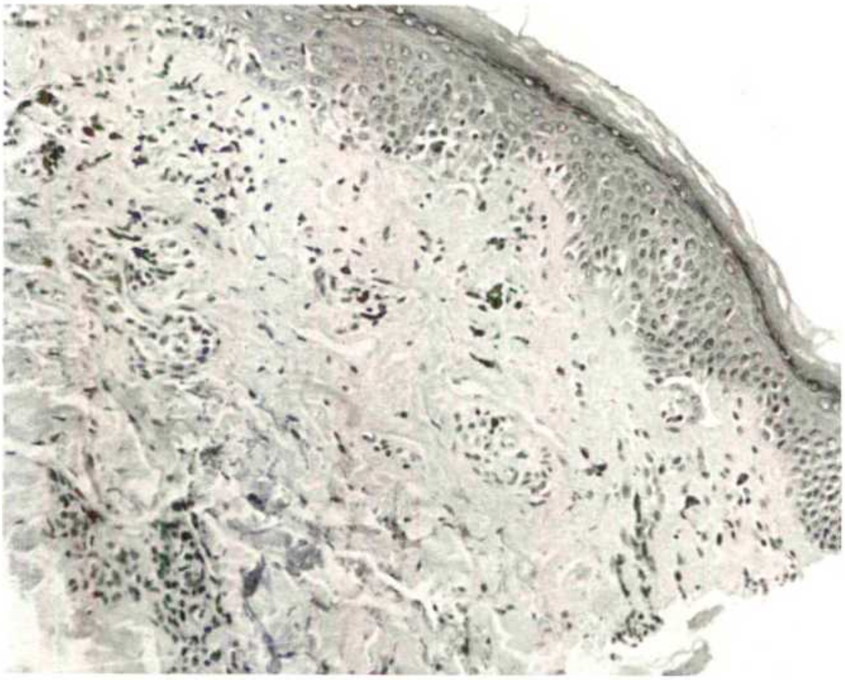

FIGURE 2. Interface dermatitis, monocytic perivascular infiltrate and pigmentary incontinence. (Haematoxylin and eosin original × 25).

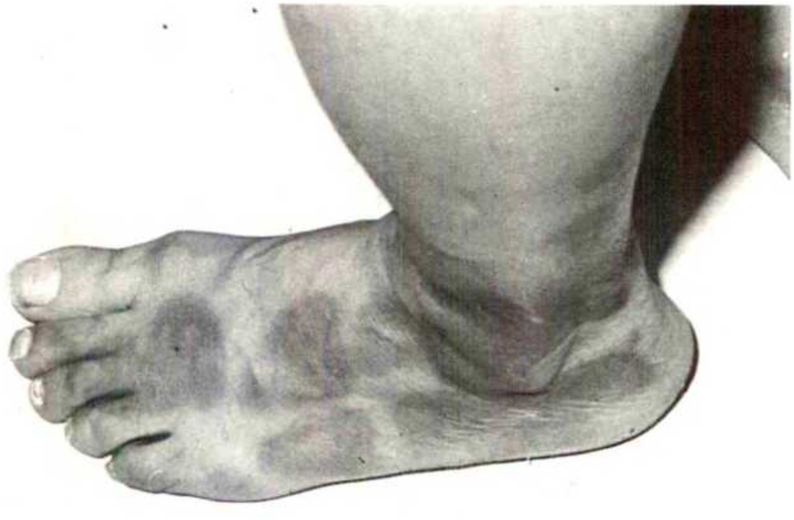

FIGURE 3. Residual pigmentation on the left foot.

clinical symptoms were found. Histological examination of a skin biopsy from a macular lesion on the buttock showed interface dermatitis with rare necrotic keratinocytes on the basal layer and in the stratum malpighii; a slight monocytic perivascular infiltrate was seen in the upper and mid dermis. Rare non-leukocytoclastic neutrophils were seen in the infiltrate. Dense melanotic pigment deposits were observed in the dermis (Fig. 2). The following were within the normal range: erythrocyte sedimentation rate, full blood count, blood glucose, serum creatinine, protein electrophoresis, hepatic enzymes, serum urea and electrolytes. A chest X-ray was normal. Clearing of the bullous eruption occurred within 15 days after withdrawal of the drug and treatment with topical antiseptics, leaving local residual hyperpigmentation (Fig. 3). Eight months later, local provocation tests using cephazolin (10% in white petrolatum, lanolin-petrolatum, Biobase–Pharminter Laboratories, and glycerin) were performed as described by Alanko *et al.*[1] and were negative.

A fixed drug eruption is diagnosed on the basis of the history and the clinical and histological findings.[2] No report of a cutaneous reaction to sodium cromoglycate has been published to our knowledge. Local provocation tests with cephazolin at previously affected skin sites were negative, but we did not dare to use an intramuscular provocation test. As no other medication had been given, we conclude that cephazolin was responsible for the eruption. Antibacterial agents have been reported to provoke fixed drug eruptions.[2,3] As far as we know this linear pattern of lesions in a fixed drug eruption has not been reported previously. Random distribution of the lesions is usual, although peculiar distributions have already been noted: thoracic lesions following the line of the intercostal nerves, central trunk lesions straddling the midline symmetrically, and symmetrical macules on the ears and lips.[4] In our case, it would seem that the linear pattern may have followed a neural distribution (S1 nerve root) rather than Blaschko's lines. The administration by intramuscular injection may have played a role in this linear distribution.

Departments of Dermatology and *Pathology
Centre Hospitalier Victor Dupouy
95107 Argenteuil, France

M.SIGAL-NAHUM
A.KONQUI
A.GAULIER*
S.SIGAL

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Cryosurgery in old world cutaneous leishmaniasis

MADAM, In an infectious disease that affects millions of individuals worldwide and in which the most widely used drug (pentavalent antimony) is described as the 'best of a bad bunch',[1] the report of Bassiouny *et al.*[2] describing near perfect therapeutic results with cryosurgery was impressive and full of promise. Using a carbon dioxide cryomachine, Bassiouny *et al.*[2] treated 30 patients with old world cutaneous leishmaniasis with a single freeze technique; all patients healed with good cosmetic results in 4–5 weeks, no relapses or complications were noted in follow-ups of 6–12 months and histopathological studies revealed that cryosurgery eradicated all parasites in less than an hour. Following this study we have utilized cryosurgery as a first choice treatment in the management of patients with cutaneous leishmaniasis attending our leishmaniasis field clinic. We wish to report that our results with cryosurgery have not been

TABLE 1. Results of treatment with cryosurgery, sodium stibogluconate and ketoconazole in cutaneous leishmaniasis

Treatment	Number of patients treated	No. of patients Healed	 Partially healed	 Failed
Cryosurgery	88	24(27%)	20(23%)	44(50%)
Sodium stibogluconate[a]	211	87(41%)	63(30%)	61(29%)
Ketoconazole[b]	310	92(30%)	136(44%)	75(26%)

[a] Sodium stibogluconate given at a dose of 10 mg/kg (600 mg maximum) i.m. daily for 14 days; results were assessed at 6 weeks.

[b] Ketoconazole given at a dose of 100–600 mg orally (according to body weight, as a single dose or in divided doses) for 4–6 weeks; results assessed at 6 weeks.

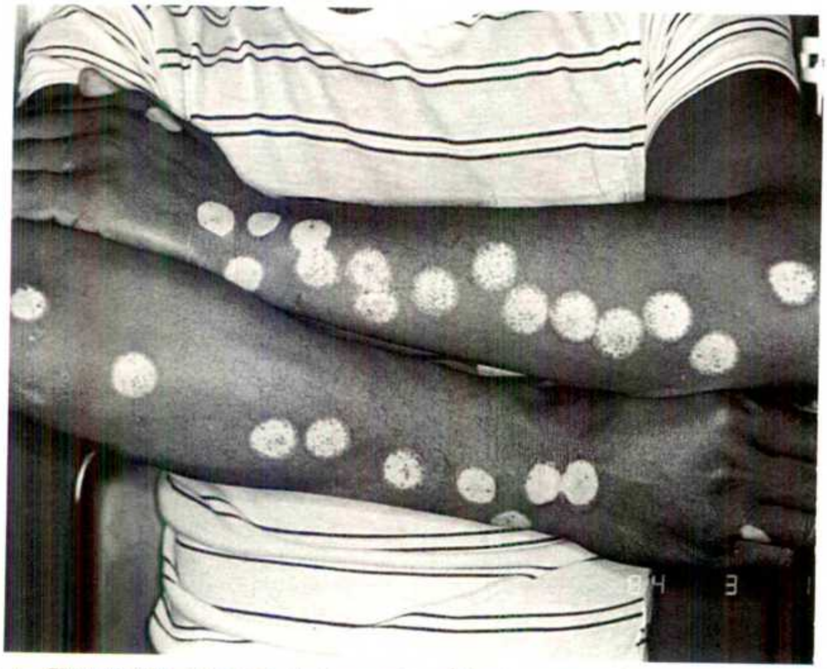

FIGURE 1. Characteristic patterned depigmentation following successful cryosurgery of multiple cutaneous leishmaniasis in a Somali patient. The patient was treated in another centre and was seen by us for the severe bullous cryoreaction that followed.

as good as those of Bassiouny *et al.*[2] and that this treatment was neither as successful nor as free from complications as suggested by these authors.

As a part of the National Leishmaniasis Research Project, we evaluated cryosurgery along with pentavalent antimony and ketoconazole as first choice treatments in leishmaniasis patients seen in our field clinic located in Al-Hassa, a known endemic focus of zoonotic cutaneous leishmaniasis. In this well studied area the parasite is *L. major* (zymodeme Lon 4), the vector is the sandfly *Phlebotomous papatasi* and the principal animal reservoir is the gerbil *Psammomys obessus.*

Cryosurgery was evaluated in an open study in previously untreated patients using the method of Bassiouny *et al.*,[2] but with two modifications; firstly we used a KRY-MED nitrous oxide cryomachine and secondly, we excluded patients with lesions larger than 2·5 cm, patients with more than five lesions, patients with lesions showing large endophytic components (iceberg lesions), and those patients who showed evidence of local dissemination such as satellite papules or lymphatic nodules. The patients were examined at weekly intervals after cryosurgery and the cryoreaction was assessed and any complications noted. The final outcome of the treatment was assessed at 4 weeks. A lesion was considered healed if it was completely resolved, partially healed if the resolution was incomplete but at least 50%, and failed if the resolution was less than 50%. Lesions showing a relapse or signs of local dissemination following cryosurgery were also considered failed.

A total of 88 patients (60 males and 28 females; with 173 nodular or nodulo-ulcerative lesions of cutaneous leishmaniasis) completed the required follow up assessment. Forty patients were Saudis, and there were 42 children below the age of 10 years. The mean duration of skin lesions (± SD) at the time of cryosurgery was 6 ± 4 weeks. The results of cryosurgery are shown in Table 1 and compared with results obtained with sodium stibogluconate and ketoconazole. The success rate of cryosurgery was found to be independent of age, sex and nationality. In a few of the patients with multiple lesions some lesions healed while others were partially healed or failed; such patients were graded according to the lesions showing the least response. No relationship was observed between the size of the lesion and the success rate in this group. However, in a pilot study we had observed a much higher failure rate in lesions larger than 2·5 cm. Relapses were observed in a few partially healed patients in whom the residual lesions enlarged (in two patients beyond pre-treatment size).

A number of complications was noted. The cryoreaction was sometimes excessive and added to the morbidity. Secondary bacterial infection was surprisingly rare. Marked depigmentation at the treated site was a constant feature. It was patterned and corresponded to the shape and size of the cryoprobe (Fig. 1). The depigmentation was most evident at 4 weeks. Repigmentation occurred in 3–6 months, usually commencing from the perifollicular zone. Cryosurgery seemed to trigger dissemination as satellite papules (11 patients), lymphatic subcutaneous nodules (one patient) and subcutaneous extension (one patient). The details of dissemination phenomena have been described elsewhere.[3,4] One patient developed persistent lymphoedema following successful treatment of a solitary lesion on the dorsum of the hand.

We were surprised at and disappointed with the low success rate of cryosurgery in our study. This contrasts not only with the results of Bassiouny *et al.*,[2] but also with the results of another study carried out in Israel[5] in which 15 patients with acute cutaneous leishmaniasis were treated with liquid nitrogen with 100% success. There are two other case reports of successful treatment of cutaneous leishmaniasis with cryosurgery.[6,7]

Besides our less impressive results, it appears that we are alone in considering that cryosurgery of cutaneous leishmaniasis is not entirely without complications. Post-treatment hypopigmentation was reported in other studies,[2,5] but was not emphasized. Scarring, not reported in other studies, was seen in most of our patients.

Our present results support Bryceson's opinion of many years ago that the various treatments of cutaneous leishmaniasis merely make up a bad bunch.[1] We contend that cryosurgery is just another treatment in this bunch and not particularly the best. An ideal treatment has as yet to be found. However, being a physical treatment, cryosurgery expands the range of treatment options available. Cryosurgery has advantages over chemotherapy in pregnant women and in patients with underlying systemic disease. We also prefer to try it first in children, especially when they have few small lesions. The relative ease, cost effectiveness and safety of cryosurgery must be weighed against the lower success rate and the possible complications when selecting treatment for a given patient. Depigmentation is less of a problem in fair skinned patients.

College of Medicine
King Faisal University
P.O. Box 2114, Dammam 31451
Saudi Arabia

Y. AL-GINDAN
R. KUBBA
A.H.S. OMER
A.M. EL-HASSAN

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A case of mycosis fungoides (cutaneous T cell lymphoma) with cold agglutinins

MADAM, We report the unsual finding of cold agglutinins in mycosis fungoides (cutaneous T cell lymphoma; CTCL). Cold agglutinins are immunoglobulin molecules, usually of IgM class, which cause agglutination of red cells on the cooling of blood. Patients may be asymptomatic, or present with haemolytic anaemia, Raynaud's phenomenon or acrocyanosis. Cold agglutinins have been described in association with a number of diseases including infections, especially with *Mycoplasma pneumoniae*, and B cell lymphoproliferative disease.[1] We report here the second case, to our knowledge, of cold agglutinins occurring in association with cutaneous T cell lymphoma.

A male patient presented in 1980 at the age of 62 with a scaly, pruritic, erythematous rash of gradual onset on the legs. The rash initially responded to emollients, but recurred and gradually became generalized.

He appeared otherwise well and at no time did examination reveal lymphadenopathy or organomegaly.

A diagnosis of mycosis fungoides was made on the basis of the clinical and histological appearances, including lymphocyte marker studies, and the gradual evolution of the condition. He initially responded to a combination of topical steroids and emollients, but gradually deteriorated over a period of 5 years. In 1985 he developed a widespread eruption of thick brownish dusky red plaques. These responded to treatment with psoralen and UVA (PUVA). He remained adequately controlled on PUVA until August 1986. Then, over a period of 2 weeks, he rapidly developed a widespread eruption of plagues and tumours on the skin. A number of the largest tumours, especially those over the buttocks, ulcerated and became painful. There was little response to isotretinoin 70 mg daily when added to PUVA, but a further partial remission was achieved by whole body electron irradiation and this was maintained by isotretinoin.

Cold agglutinins were first noted in a routine blood film in January 1986, and were subsequently noted in every blood film. The cold agglutinin titre, measured in August 1986 was 1 : 1024 for cord group O cells and 1:512 for adult O cells at a temperature of 4°C. Both were negative at 37°C. The antibody was, consequently, designated as having anti-Pr specificity.[2] The full blood count persistently showed no evidence of haemolysis, nor were any circulating Sézary cells noted. *Mycoplasma* serology was negative. There were no symptoms ascribable to cold agglutinins.

Cold agglutinins associated with B cell lymphoproliferative disease have been well described[1] and usually display monoclonal characteristics including electrophoretic homogeneity. The occurrence of cold agglutinins in a patient with Sézary syndrome, a leukaemic variant of CTCL, has been reported.[3] There are no previous reports, to our knowledge, of cold agglutinins occurring in uncomplicated mycosis fungoides. It would seem likely that the phenomenon is due to stimulation of B lymphocytes by the malignant clone of T helper/inducer cells.

It is possible that this isolated case is simply a chance association, however, mycosis fungoides and the presence of cold agglutinins, in particular with anti-Pr specificity, are both relatively rare. Cold agglutinins may, therefore, represent a previously unrecognised feature of mycosis fungoides. If so, this should be established, both to assist with the diagnosis of the skin condition, which can be difficult in the early stages of the disease, and to prevent unnecessary investigation.

Departments of Medicine (Dermatology) and *Haematology,
University Hospital of Wales,
Heath Park, Cardiff CF4 4XN, U.K.

J.Berth-Jones
J.Holmes*
R.Marks

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Thiabendazole-induced erythema multiforme with lesions around melanocytic naevi

Madam, We describe a rare cause of erythema multiforme, with the unusual feature of lesions encircling pre-existing melanocytic naevi.

A 37-year-old woman developed a widespread pruritic erythematous eruption preceded by peri-orbital oedema, conjunctival injection, dysuria and painful oral ulceration. Sixteen days prior to onset of the rash she had taken a course of six 500 mg thiabendazole tablets in two doses for the treatment of presumed threadworm infestation. This agent had been prescribed on medical advice, but was obtained as an 'over the counter' preparation. On examination she had many coalescent annular urticated erythematous lesions, particularly on the trunk. The day after she first attended the out-patient department, she became pyrexial with rapidly increasing peri-orbital oedema and general malaise and was admitted to hospital. Her rash had worsened and involved the face, arms and legs; lesions had cleared from some of the areas previously involved, leaving purpuric staining. Some lesions had early central bulla formation. It was noted that many of the lesions on the limbs encircled pre-existing benign melanocytic naevi. The clinical diagnosis of erythema multiforme was supported by typical histological features in a skin biopsy, but all other investigations were normal or negative. Treatment was with oral prednisolone.

Thiabendazole is an imidazole compound which is used medically as an antihelminthic agent, but which also has a wide spectrum of activity against plant pathogenic fungi and is, therefore, important in agriculture. Skin eruptions due to oral thiabendazole are infrequent and are usually mild and transient. Most have been pruritic urticarial or maculopapular rashes occurring within hours or days of treatment, and lasting a few days.[1–5] Our patient developed severe erythema multiforme 16 days after a course of thiabendazole, and no other likely cause of this eruption could be demonstrated. Although erythema multiforme is included as a possible side-effect in the manufacturer's data sheet, no such cases have been reported to Merck, Sharpe and Dohme in the U.K. (Dr K. Condon, personal communication). A case of toxic epidermal necrolysis was reported by Robinson and Samorodin,[6] but we are not aware of any other reported cases of eruptions in the erythema multiforme spectrum.

An interesting feature in our patient was the tendency for lesions to be centred around pre-existing melanocytic naevi. This is distinct from the cases of eruptive naevi reported following erythema multiforme or Lyell's syndrome.[7–9] A papulosquamous eruption around naevi with some features suggestive of pityriasis rosea has been reported by Meyerson,[10] and halo eczema may occur around naevi.[11] There were no clinically detectable changes in the naevi in our patient, and no new naevi developed after her erythema multiforme resolved.

Although severe erythema multiforme due to thiabendazole is rare, commonly reported side-effects of oral thiabendazole are nausea and giddiness.[12] As these occur in up to 50% of patients and may be severe, we suggest that use of this drug for medical indications should be on a prescription rather than an 'over the counter' basis.

Department of Dermatology
Royal Victoria Infirmary
Newcastle upon Tyne, U.K.

F.HUMPHREYS
N.H.COX

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Pseudoepitheliomatous micaceous and keratotic balanitis

MADAM, The account by Beljaards *et al.* (*British Journal of Dermatology* 1987; **117:** 641) relating pseudoepitheliomatous micaceous and keratotic balanitis (PMKB) and verrucous carcinoma is of great interest. Verrucous carcinoma is probably associated with HPV infection, and may indeed be identical with the giant condyloma acuminatum of Buschke and Lowenstein; investigation into this aspect might be revealing. There appears to be a curious overlap between PMKB, verrucous carcinoma, HPV infections, penile horns, and also lichen sclerosus et atrophicus (LSA). Willscher *et al.*[1] described penile horns in two patients; one had associated condyloma acuminata while the other is said to have had balanitis xerotica obliterans (the main cause of which is LSA), and a verrucous carcinoma. Lowe and McCullough[2] noted the 17 cases of penile horns described in the literature, commenting that about one third were associated with malignancy (in their own case invasive squamous cell carcinoma). They pointed out also that penile horns tend to develop on longstanding phimosis (as does PMKB, while LSA is also a recognized cause of phimosis in adult life). The case of Bart and Kopf[3] included in their survey, was of a penile horn in association with PMKB. Frances *et al.*[4] described warty growths clinically like giant condylomata acuminata, and histologically showing koilocytosis, but felt to be unique because of striking dyskeratosis, in two patients in association with penile LSA.

These comments are prompted by the example of a patient who was thought on clinical and histological grounds to have PMKB, a diagnosis accepted at the Section of Dermatology meeting at the RSM in 1966. Over the next few years, however, the appearance came to resemble LSA, with eroded and perhaps malignant areas. These latter diagnoses were finally confirmed histologically. There was intra-epithelial neoplasia, and invasion could not be excluded. Marked koilocytosis was present, but techniques of *in situ* hybridization to detect HPV DNA were not available. On clinical and histological grounds the diagnosis of penile intra-epithelial neoplasia perhaps with a squamous cell carcinoma, rather than of a verrucous carcinoma, was made and the lesion was, therefore, treated with radiotherapy on the advice of the urologist. Two years later the patient remained well with a soundly healed penis showing only signs of LSA.

Perhaps one might add other questions to that of Beljaards *et al.* Could penile horns and PMKB be related to each other and to LSA, could they all indeed be aspects of LSA, and is HPV concerned in the development of carcinoma in these patients, whether of verrucous, intraepithelial or invasive squamous type?

St Mary's Wing,
Whittington Hospital,
London N19 5NF, U.K.

C.M.RIDLEY

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Amenorrhoea during treatment with isotretinoin

MADAM, Reports of menstrual irregularities in patients treated with isotretinoin or etretinate are uncommon and, as many patients are treated concurrently with an oral contraceptive, they may be under-reported. Amenorrhoea is a particularly important side-effect as it creates concern about the possibility that the patient might be pregnant. The following case illustrates this problem.

A 14-year-old girl had a 5-year history of severe nodulo-cystic acne which had not responded to therapy with two tetracycline drugs. She was commenced on isotretinoin (Roaccutane®, Roche Products Ltd) after detailed explanation of likely side-effects, and was supplied with the patient information sheet prepared by the manufacturers. The risk of teratogenicity and pregnancy was discussed with the patient and her mother, who both stated that no oral contraceptive treatment was required. The dosage of isotretinoin was 25 mg daily for the 1st month and 40 mg daily thereafter. Treatment was discontinued after 10 weeks as the patient admitted that she had not menstruated since starting isotretinoin therapy, although she had had regular menses every 4 weeks for the preceding 3 years. Pregnancy testing was negative, but more detailed endocrinological investigation was not performed as menstruation occurred about 10 days after stopping isotretinoin and was regular subsequently. Her acne had improved considerably during the 10 weeks of treatment.

The normal menstrual pattern in this patient before and after treatment with isotretinoin suggests that the amenorrhoea for two cycles during treatment was related to the drug. Menstrual irregularities associated with isotretinoin are uncommon with the usual 4-month course of treatment, but occurred in all five patients treated with long-term low-dose isotretinoin by Edwards *et al.*[1] Six of 53 female patients (15%) treated by Bruno *et al.*[2] reported abnormal menses during a 4-month course of treatment, the symptoms generally being of a 'late' or 'skipped' menstrual period. One of these patients had amenorrhoea for two cycles. In the U.K. the Committee on Safety of Medicines has only received five reports of menstrual irregularity in patients treated with isotretinoin, of which only one patient was reported to have amenorrhoea (CSM, personal communication). Side-effects of isotretinoin, etretinate and vitamin A are similar, and it is therefore of interest that oligomenorrhoea and decreased libido have been reported in a female patient treated with etretinate[3] and that menstrual disturbance is an occasional feature of hypervitaminosis A syndrome.[4]

Because of the requirement for adequate contraception during oral retinoid therapy, many sexually active female patients may be treated concurrently with an oral contraceptive agent. The effect of isotretinoin on sex hormone levels in patients taking oral contraceptives was studied by Orme *et al.*[5] and, although there was a fall in plasma ethinyloestradiol levels in two of their patients, no systematic reaction between isotretinoin and oral contraceptives was demonstrated. This suggests that oral contraceptive agents function normally in most patients taking isotretinoin. Whilst reassuring in terms of concern about teratogenicity, it is possible that regular contraceptive withdrawal bleeding would mask menstrual irregularities due to oral retinoid therapy. Menstrual disturbance may therefore be a more frequent side-effect of oral retinoid drugs than most studies would suggest.

Department of Dermatoloy
Royal Victoria Infirmary
Newcastle-upon-Tyne NE1 4LP, U.K.

N.H.Cox

ACKNOWLEDGMENTS

I am grateful to the Committee on Safety of Medicines for permission to cite details of their adverse drug reaction data.

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News and Notices

4th Immunodermatology Symposium

The 4th Immunodermatology Symposium will be held at the Sonesta Hotel, Amsterdam, The Netherlands, on 21–23 September 1989. Free communications in all areas of immunodermatology are encouraged.

For further information please contact QLT Convention Services, Keizersgracht 792, 1017 EC Amsterdam, The Netherlands.

Practical Dermatology Course for Registrars and Senior Registrars

A practical dermatology course for registrars and senior registrars will be held in Oxford, U.K. on 27–28 October 1988. The course, which is open only to trainees, will entail instruction on rational investigation and management in the fields of vascular and lymphatic diseases, disorders of hair, and the rational use of immunofluorescence. Cryosurgery and infra-red coagulation will be demonstrated. Each section will comprise lectures and clinical demonstrations, involving patients wherever possible. For further details please apply to: Dr R.P.R.Dawber, 'October Course', Department of Dermatology, The Slade Hospital Headington, Oxford OX3 7JH. Telephone: 0865 64841 ext 499.

International Foundation of Dermatology

The aims of the International League of Dermatological Societies according to its rules and regulations, is to encourage the advancement of dermatology; to promote personal relations among the dermatologists of the world; to represent dermatological interests in other international organizations, and to hold international congresses on dermatology. For several decades the International Congress of Dermatology has occupied most of the attention of the members of the International Committee, and it has been an anxiety for successive members of the committee to realize that they are perhaps doing too little for the developing world. Stimulated by Dr Darrell Wilkinson, the idea of an International Foundation for Dermatology came to fruition when the International League of Dermatological Societies at the International Congress in Berlin agreed that there should be such a Foundation to further and develop dermatology throughout the world. The Foundation is a subsidiary of the International League of Dermatological Societies, and knowing of its existence, several national societies and many individuals have already contributed finance to support its objectives. All such donations will be used for the furtherance of its aims. The cost of setting up the Foundation will be covered by donations from a sum donated specifically for that purpose by the members of the International Committee of Dermatology. The Board of Directors of the Foundation, chaired by Dr Alfred W. Kopf, U.S.A., and supported by regular members of the International Committee, Dr Terence Ryan, Great Britain, Dr Ramon Ruiz-Maldonado, Mexico, together with the President of the International League of Dermatological Societies, Professor Klaus Wolff and the Secretary General, Professor Stuart Maddin, and two co-opted members, Dr Henning Grossman and Professor Francisco Kerdel-Vegas, agreed at a meeting in December on the following missions for the International Foundation for Dermatology:

The Primary Aim

The International Foundation for Dermatology, which serves under the aegis of the International Committee of Dermatology of the International League of Dermatological Societies, has as its primary aim to 'Aid dermatology and patients afflicted with diseases of the skin in developing countries.' These aims are to be carried out under the guiding principles of resource allocation to manage these preventable, curable, and common diseases of the skin which affect so many in developing countries.

The following are the principal missions of the International Foundation for Dermatology:

Mission 1. Promote dermatological education and communication in developing countries at national and international meetings.

Mission 2. Document the current status of dermatological manpower and the burden of dermatological disease worldwide.

Mission 3. Establish official relationships with the World Health Organization.

Mission 4. Promote dermatological education and training in developing countries.

Mission 5. Promulgate collaborative programs between institutions from developed and developing countries of the world.

Mission 6. Support the establishment of fully-fledged departments of dermatology in at least one medical school in each country.

Mission 7. Improve delivery of dermatological care in developing countries.

Mission 8. Develop a cadre of experienced dermatovenerologists willing to serve on a short- or long-term basis as visiting teachers, lecturers, advisors, or practitioners of dermatology.

Mission 9. Develop basic pharmacopeas for primary dermatological care and provide dermatological pharmaceuticals for the needy.

Mission 10. Support research geared toward better understanding of the pathogenesis and natural history of dermatological diseases of major public health concern in developing countries.

The formal business of incorporating the Foundation to enable it to pursue its aims and receive donations without incurring tax, is now in progress. Societies and dermatologists throughout the world will be kept in touch with all developments in the expectation that they will both support and benefit from its aims. Further details available from Dr T.J.Ryan, Consultant Dermatologist, The Slade Hospital, Headington, Oxford OX3 7JH, U.K.

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